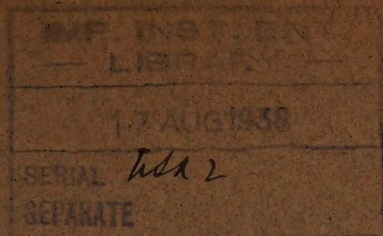


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DUTCH ELM DISEASE

Graphium ulmi

G. P. CLINTON AND FLORENCE A. McCORMICK



Connecticut
Agricultural Experiment Station
New Haven

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DUTCH ELM DISEASE

Graphium ulmi

G. P. CLINTON AND FLORENCE A. MCCORMICK

Introduction

THE DUTCH ELM DISEASE is the common name of a trouble first described from Holland in 1921, and nine years later identified in the United States. Early in its history this disease was found to be caused by a fungus new to science and called *Graphium ulmi*, *ulmi* being the generic name of the host upon which it occurred. Later it was discovered that this *Graphium* is only a stage in the life history of the fungus and that its mature stage is an Ascomycete of the genus *Ceratostomella*. Therefore the fungus is now usually called *Ceratostomella ulmi*. More recently still the genus *Ceratostomella* has been divided, and the elm fungus has been placed by Nannfeldt (39) under the genus *Ophiostoma*, becoming *Ophiostoma ulmi*. So we have at least three scientific names for the Dutch elm disease. As the imperfect stages of the fungus cause the injury to elm trees, in this laboratory we use the one name *Graphium ulmi*, rather than its saprophytic asco stage, though another imperfect stage, known usually as *Cephalosporium*, is associated with the *Graphium* but is less distinct in its fruiting structure.

The Dutch elm disease was first found in this country in Ohio in 1930. After several preliminary searches it was discovered in Connecticut a few years later. Because of the seriousness of the disease and the importance of the elm as a shade tree, the Station began to study the fungus and to coöperate with the United States Department of Agriculture in its control. So far control has been largely by extermination of all infected trees and sanitation measures. Only 136 cases have been found in Connecticut¹ and these were almost entirely in the southwestern part of the State adjacent to the badly infested area of New York and New Jersey. Therefore it seemed likely that this State could best serve as a barrier against the spread of the trouble into New England where the elm is most widely and commonly established as a shade tree.

This bulletin treats chiefly the scientific side of the problem rather than the control measures that have been used in Connecticut. It deals first with the history of the trouble in Europe, where most of the scientific facts were discovered; then with the introduction of the fungus into the United States, where further researches were and are being made; and finally with the researches in this laboratory. A general statement of the character of the fungus and its effect on the elm will be found on pages 720-723.

HISTORY OF GRAPHIUM IN EUROPE

Discovery. Shortly after the World War it was noticed that various trees, and more particularly the elms, were dying in unusual numbers in Holland, Belgium and France, and later in Germany. At that time Tubeuf

¹ Since the above was written (the last of April, 1935) the number has increased to 235 by the middle of October, when the paper went to the printer.

(65) of Germany published an article on the death of elm branches in the springs of 1918 and 1920. For a while it was thought that this was the first article relating to the appearance of the Dutch elm disease. Actually, Tubeuf described an excessive development of the fruiting condition of the elms (usually occurring each alternate year) which robbed the leaf buds of their normal development and often resulted in the death of the branches or at least a scarcity of their foliage. We saw a similar local injury here in Connecticut in the spring of 1934, following winter and drought injury to street trees. It was in Holland, however, that the real cause of the trouble was first determined. Hence the common application—the Dutch elm disease.

Reports from Different Countries

Holland. So far as we have learned, the first definite reference to the cause of the Dutch elm disease came from Holland and was written by Dina Spierenburg (63) in February, 1921. In this article the writer mentions injury to elms as early as 1919, or even earlier, as shown by injury to the growth rings of previous years. She made an examination of insects and fungi in the wood and bark as possible causes. She mentions a definite insect called *Eccoptogaster scolytus*, now known as *Scolytus scolytus*, and various fungi, especially *Cephalosporium acremonium* and *Graphium penicillioides*, in connection with this trouble. An accompanying photograph pictures a good cross-section of an elm twig showing the characteristic brown spots in the recent wood rings. A year later the same author (64) published a much more extensive article with several illustrations showing the effect of the disease on the trees and a list of the places where the trouble had been found in Holland at that time. Photographs of *Graphium* and *Cephalosporium* and the growth of the latter in Petri dishes illustrate the text. Apparently Miss Spierenburg had found the cause of the trouble, though she did not consider it a new species of *Graphium* and did not definitely limit the injury to this single fungus.

In December, 1922, Dr. Marie Schwarz (57), a graduate student of Dr. Johanna Westerdijk of Baarn, gave a more definite account of the trouble, associating it with a new species of a fungus which she called *Graphium ulmi*. She also made studies of other fungi that she obtained from diseased elms. Her infection work, made chiefly with twigs, was not very convincing in proving this *Graphium* as the sole cause of the elm disease, but the main results of her investigations played an important part in finally establishing the cause.

Dr. Christine Buisman (10) began writing on this disease in 1928 and her last article (15) appeared in 1935. Only eight of her more than a dozen papers are listed in our bibliography, but she made a more continuous study of the trouble from all angles than any other investigator. Her important paper of 1929 (11), with that of Doctor Westerdijk (70), had much to do in clearing up this *Graphium* disease from other troubles that were produced in the elms by bacteria, *Verticillium*, *Phomopsis* and other fungi. Especially important were her inoculations of young elms with *Graphium*. Her later papers (12, 15) deal further with these inoculation experiments. She discussed the susceptibility or resistance of various species of elms to the *Graphium* fungus; the finding (12, 13) of the asco

stage, *Ceratostomella ulmi*, first in artificial cultures on elm twigs inoculated with plus and minus strains of the fungus and then in nature; the distribution (14) of the disease in other countries; and the advisability of planting resistant species of elm, especially those of Asiatic origin.

While in this country, Doctor Buisman helped to identify the disease from Ohio. She also confirmed our belief that the elm fungus that we had at that time under investigation in Connecticut was not the true Dutch elm disease.

In 1934, Dr. Maria Ledebøer, also of Baarn, published a long paper (33), "A Physiological Research upon *Ceratostomella ulmi*." She used artificial cultures of the fungus in various media, including sugars, peptones, etc., for carbon and nitrogen, as well as various salts (of potassium, sodium, calcium, magnesium, copper, zinc, iron, phosphorus, manganese, mercury) and tannin, to determine the effect of these, of temperature and light, and of pH, on growth of the fungus. Her results in part showed: Optimum temperature necessary for growth was about 25°C; light, not absorbed by ordinary glass, was best for development of coremia; the best pH was between 6-7; 5 per cent saccharose, and eventually glucose, gave the maximum growth as did several other organic substances. The results with mineral salts varied with the percentages used; corrosive sublimate used in small amounts was a stimulant; tannin was not helpful; cellulose was not made use of; peptone, as a source of carbon, was not necessary.

Besides these authors, there were a few other Dutch scientists who made studies of the bark beetles, especially *Scolytus scolytus*, and of how these insects spread the fungus among elms. These persons will be mentioned later.

France and Belgium. About the time of its discovery in Holland, the Dutch elm disease was also found in nearby countries and reference was frequently made to the work in the Netherlands. Guyot (31) in France, in the late fall of 1921, reported a serious trouble of elms in Picardy. It was first observed in 1918 and was apparently of this nature and not directly due to war gases as some claimed. Marchal and Foex in 1921 also treated of this disease, and Foex (18) in 1922 refers to the article published by Miss Spierenburg and gives northern France, Belgium, Holland and perhaps Germany as its distribution in 1918. Since then other articles describing the disease have been published by Dufrénoy (17), Guinier (29, 30), Demorlaine (16), Arnaud and Barthelet (3) and others.

We have not seen much Belgian literature on the subject, but accounts of it were written by Biourge, Marchal (38), and Gallot. Apparently these writers depended largely on the scientific work published in Holland. Manil, in his letter quoted later, states that the disease first appeared in the northern part of Belgium in 1919 and rapidly spread toward the South.

Germany. We do not know who first discovered this disease in Germany, but between 1924 and 1928 a number of investigators wrote concerning it as follows: Pape, in 1924; Brussoff, Höstermann, Noack and Lüstner, in 1925; Gante, Falck, in 1926; Graf von Linden and Lydia Zenneck, Wollenweber, in 1927; Stapp, Uphof, in 1928. According to the letter from Richter, quoted later, the trouble appeared in West Germany in 1924; Pape (41) records examinations made for elm troubles in Bonn

in 1921; Tubeuf states that it was known in South Germany in 1927; Prell, that it was first known in Dresden in 1928. Höstermann and Noack also wrote an article in 1925 concerning the death of elms on the Rhine.

It was the article published by Brussoff (6) in 1925, however, that stirred up trouble. This author claimed the death of elms was due to a new species of bacteria, *Micrococcus ulmi*, which he described. He isolated this germ from injured trees and claimed to have reproduced the injury to healthy elms by inoculating the same. He also stated in 1926 that he had isolated this same germ from maple and linden (7) and later from beech and poplar (8). Countess von Linden and Lydia Zenneck (35, 36), in 1927 disputed his claim and agreed with the work of Dr. Schwarz of Holland that the injury was caused by *Graphium ulmi*.

Investigations of Wollenweber and Stapp (77) in 1928, who worked both with *Micrococcus ulmi* and with *Graphium ulmi*, showed that the latter was the real cause of the Dutch elm disease and the insect *Scolytus scolytus* was an important factor in its spread. Wollenweber, (74, 75, 76), both before and since 1928, made other contributions along the same line, especially by his inoculation experiments with *Graphium ulmi* on elms. In the 1928 article he seems to have been the first to suggest *Ceratostomella* as a possible asco stage.

Other early writers on this disease in Germany were Metzger (40) in 1927, Mary Wilson (73) in 1929, and Prell (53) in 1930. Metzger, in his article concerning treatment for the Dutch elm disease, refers to Ilisch's method of inoculating trees with liquid taken from diseased trees and reports success for elms so inoculated. Apparently this method, however, was not successful elsewhere.

There has also been some controversy in Germany as to whether all of the injury to elms is due to this *Graphium* fungus or whether drought, winter injury and other unfavorable conditions have had their part in the death of the elms and other trees. Apparently Pape (41), who failed in his infection experiments with *Graphium* as a cause, Höstermann and Noack, Lüstner, and later Lüstner and Gante (37) and Knoll have written along this line. In 1935 Lüstner and Gante made the following (translated) statement in the conclusion of their article (37, p. 93):

For the complete understanding of the graphiose and the related transient or death-dealing disease of infected elms, it would seem that the variability of the tree should be considered. This variation may rest on the degree of susceptibility of the different elm families, sorts, or individuals. Many investigations and proofs indicate that outside influences, especially drought, may make the elms susceptible to the disease. Various infection experiments in Berlin-Dahlem, in Holland and Geisenheim have shown that the fungus must find the plant in a certain condition in order to destroy it. The early fungous inoculations in these cases led only to a transient infection. The following year the elms were found to be healthy.

Tubeuf (67), in his 1935 article which was printed in the same magazine as that of Lüstner and Gante, gave an extended account of the investigations of the disease in different countries, particularly in Germany and Holland. He also raised various questions that he believed had not yet been answered besides describing his personal investigations.

England. In 1927 Wilson (71) of the University of Edinburgh, Scotland, was one of the first to discuss the possible danger of this disease if it started in Great Britain. Later he and Mary J. F. Wilson (72) in

Gardner's Chronicle, 1928, recorded finding an outbreak near London, England, in July, 1927, and stated that the disease was apparently present in 1926.

Dr. Mary Gregor (28), of Edinburgh, in 1932 made a special study of *Ceratostomella pluriannulata* which was associated with *Graphium ulmi* on an elm stump. At first she thought this was the asco stage of the *Graphium*. She found it to be heterothallic, as did Doctor Buisman later, for *Ceratostomella ulmi*. Doctor Gregor's study of this other species, however, throws light on the development of the asco stage of these fungi whether placed under *Ceratostomella* or *Ophiostoma*. The elm disease has not yet been found in Scotland.

Since its discovery in England, Peace (42, 43), of Oxford and of the Imperial Forestry Institute, has made most of the preliminary investigations on the spread of *Graphium* and its effect on elms in England. By 1931 it had been found in 38 counties. A letter from Peace, quoted later, gives further details for 1935. Apparently in England no serious measures for the eradication of the disease have been taken and some persons believe that the fungus is not always fatal to the infected trees. The following statement is taken from an article by Peace (45) written in October, 1935:

"The disease is still making slow progress in nearly every area, and it has very nearly reached the degree of severity recorded in 1931. The total number of cases seen this year was 1600 and 800 dead elms. In the past five years the total number of cases and of dead elms seen was as under:

Year	Diseased	Dead	Period of Survey
1930	2000	200	Fortnight
1931	3000	400	Fortnight
1932	2500	700	Three weeks
1933	2000	800	Three weeks (part in Scotland)
1934	2400	1000	Sixteen days

"Totalling the number of cases and dead trees in those areas which are truly comparable, 1935 shows an increase of 220 on 1934, an increase of 310 on 1933, an increase of 450 on 1932, but still a decrease of 70 on 1931. In some cases dead trees may have been removed and no allowance was made for them, so on those grounds the decrease of 70 may be ignored, and the disease said to have reached the 1931 level again.

"The disease has not been reported from any fresh counties this year

"As last year in some places the disease has increased considerably, in others it has made little progress, while in a few it seems to be gradually dying out"

The following year, October, 1936, Peace made a report to the Forestry Commission from which we quote the final paragraph:

"The progress of the disease is still so irregular that it is impossible to prophecy the future of the elm in England. At the present rate it will be very many years before the elm becomes uncommon, but it seems likely that unless the course of the disease radically alters, or unless many of the elms still unaffected prove to be resistant, eventually the elm will lose its position as one of the most important components of the English landscape."

Butler, of the Imperial Mycological Institute of Kew, has had much to do with reviewing literature on this subject. Recently the Division of Forest Pathology of the United States Department of Agriculture sent Dr. J. S. Boyce of Yale University to England and he selected Oxford as a place for the United States Laboratory for further study of this trouble in Europe.

Italy. Another European country, where the elm disease has appeared and which has produced more or less literature, is Italy. The Italian writers, as far as we have learned, have been Sibilìa, Petri, A. Goidanich and G. Goidanich, and such outsiders as Reinboth and Buisman. The disease was first reported in June, 1930, by Sibilìa (58) from the province of Plodena, though according to Ansaloni's letter, reported here, Professor Drachetti states it was found 20 miles west of Bologna in the summer of 1929.

Sibilìa (59), in the same year that his first paper was published, wrote a longer article on the fungus and its injury to elms. Other articles by him in the same publication (Bol. R. Staz. Patol. Veg.) have appeared in 1932 (60), 1933 (61), and 1935 (62). In the last he treats of the resistance of an Asiatic species, *Ulmus pumila*, and especially of its variety *pinnatiramosa* to *Ceratostomella ulmi*. He states that the native species of elms in Italy are very susceptible and that some of the Asiatic species, when inoculated, show more or less infection but less than the native. The resistant species and its variety have now been fairly well distributed in Italy with satisfactory results so far. He also treats of grafting *U. pumila* on various species of elms to determine resistance to this disease.

Petri also published short articles concerning the trouble in Italy in 1931 (46) and 1932 (47) and has a preface to G. Goidanich's recent article mentioned later.

One of the best illustrated articles that we have seen on the Dutch elm disease is that of Athos Goidanich and Gabrielle Goidanich (23) of Bologna. Their photographs show the appearance both of the *Graphium* stage of the fungus and its injury to elm twigs, and of the *Scolytus* beetles and their injury to the wood and twigs. The first author deals with the insects. He finds a different species of *Scolytus*, *S. sulcifrons*, as the chief insect carrier in Italy. He also mentions a number of other insects found on elms. Gabrielle Goidanich is responsible for the part of the paper dealing with the fungus. They also published a popular article on the disease in 1934 (24). In another article (27), published in 1935, concerning the genus *Ophiostoma*, G. Goidanich gives details concerning a new species found on pear which he calls *Ophiostoma catonianum* (*Graphium pirinum* as its conidial stage) that throws considerable light on this genus to which Nannfeldt¹ has recently referred the Dutch elm disease. G. Goidanich² also has a very recent article, published in 1936, that further deals extensively with the death of elms and *Graphium ulmi*.

One of the incidental complaints in Italy has been the loss of the elms as supports for grape arbors. Use of the more immune Asiatic species of *Ulmus pumila* and its variety seems to be the solution, since large trees are not necessary for these supports. Recently there has been discussion, Goidanich (25, 26) and Reinboth (54), of the serious trouble of Burbank plums that has appeared in Italy, especially in localities where the Dutch elm disease has been bad. At least one writer has suggested that *Graphium*

¹ Nannfeldt (39) in his article on page 408 calls the fungus "*Ophiostoma ulmi* (Buism.) Nannf., n. comb. (*C. ulmi* Buism.)." On page 404 he wrote "The distinguishing features of the genus *Ophiostoma* are the long ostiolar beaks, composed of parallel hyphae and terminating in a 'crown' of hyaline cilia, as well as the small deliquescent asci and the minute hyaline continuous spores shaped like the sections of an orange." Goidanich's photomicrographs show the fungus to be of this nature as do our investigations of the mature stage of *Graphium ulmi* presented later.

² La "moria dell' olmo" (*Graphium ulmi*). Sperimentatore della R. Staz. Patol. Veg. Roma: 1-134, f. 1-44, 1936.

ulmi is a possible cause, but without sufficient reason. Further information concerning the trouble in Italy may be obtained from the letters we publish in the addenda.

Switzerland, Czechoslovakia, Roumania, Yugoslavia. The literature on elm troubles in these countries is apparently rather limited. At least we have seen little of it and have learned of few authors who have written concerning the elm disease. The reason may be that *Graphium ulmi* was discovered in these countries later than elsewhere. Other possible reasons may be that the disease failed to ruin the trees, except in restricted localities, that the elms were scarce or were more or less immune to the disease. This last is said to account partly for its scarcity in eastern and northern Germany, as opposed to its prevalence in the western and southern regions of that country. However, the Dutch elm disease has been found in all four countries and in some districts has caused serious injury.

In Switzerland, apparently, it has not been so serious as in the neighboring countries of France, Germany and Italy. It is also somewhat doubtful as to when it first appeared there. Lendner (34) in 1932, whose article is the only one we have seen from Switzerland, reports he found it near Geneva at that time. He gave a photograph of an injured tree. In his letter to us he states that he believes it dates back to 1922. Doctor Buisman wrote that she and Professor Westerdijk saw it there in 1929 and she cites an article by Grossman who apparently reported it in 1932. Badoux, in a letter given here, states that the number of elms in their forests is so limited that no serious outbreak is likely to occur.

The same doubt exists as to the exact time when the Dutch elm disease made an appearance in Czechoslovakia. In their article, Kalandra and Pfeffer (32) give 1931 as the date of its discovery and they also write (translated) as follows:

According to the present observation the *Graphium* appears most in the parks and promenades of our cities (Prague, Poděbrady, Brunn, B. Štiavnica). Frequently it is found in the woods along the streams. . . . On the Hungarian border it exists along the Krupnica . . . and in Carpathian Russia in the vicinity of the river Latorica in the neighborhood of Mukacëvo. . . .

From the material from different localities in the year 1933-34, the rings of the years 1931 to 1934 were found infected. In certain cases from J. 1930 (Hodonin) and from J. 1929 (Obřístvi) infections were found in the annual rings back to 1914. The infection of the rings in 1931 was the same in Böhmen and Mähren as in Slovakia. . . . For the rapid spread of the disease, the bark beetles are the most important carriers.

These writers include illustrations showing the engravings made on elm wood by four species of *Scolytus* and two species of other genera. They also mention injury by several other species of bark beetles. Three species of elm, *Ulmus campestris*, *U. effusa*, and *U. montana*, are reported as attacked by the *Graphium*. Further information concerning the trouble in Czechoslovakia is given in the very interesting letter by Peklo cited later.

We have seen no literature concerning the Dutch elm disease in Yugoslavia but interesting information is given in the letter, *q. v.*, received from Skorić of Zagreb, who observed it there in 1928.

Alexandri and Savulescu (56) apparently are the scientists who have written concerning the disease in Roumania, but we have seen neither of

their publications and only short reviews of Săvulescu's papers.¹ However, a letter, *q. v.*, from him gives us some of the essential facts. It was written in English by his assistant Doctor Aromescu, whom the senior writer met at Amsterdam in September, 1935. This letter states that the Dutch elm disease was first found in Roumania in 1929 but its first occurrence could not be established.

Austria, Hungary, Poland, Portugal.² While some literature on the presence of the Dutch elm disease has come from these countries, we have not seen the original articles. Our data is taken from reviews by the English *Review of Applied Mycology* and from references in various articles such as that by Miss Buisman (14), "The Area of Distribution of the *Ceratostomella* (*Graphium*) Elm Disease". Our letter soliciting information from some of the writers in these countries has not been successful in obtaining further material.

In Austria, the writers have been Schimitschek in 1927, Köck in 1928, Fischer in 1931, and probably others. The disease was first discovered in 1926, apparently by Schimitschek (Wollenweber, Buisman). Fischer in 1931 reported that many elms in Vienna had succumbed to an attack by the fungus (Buisman).

In Hungary the only reference we have to the disease is that made by Doctor Buisman (14, p. 42) who states: "Professor Westerdijk received some twigs of elms growing in Hungary and apparently attacked by the elm disease. It was an easy matter to isolate *Graphium ulmi* from these twigs."

The presence of the disease in Poland in 1928 is based on Sallmann's article in *Gartenwelt*.³ He states the great death of elms not only due to *Graphium* but to other fungi, inadequate nourishment, factory fumes, etc.

We depend on Doctor Buisman's statement that the disease has been found in Lisbon, Portugal, since she writes in the previously mentioned article, p. 40: "I saw Doctor Loureiro Ferreira's cultures and the infected wood and I could confirm the presence of *Graphium ulmi* in Portugal."

Insects as Carriers

From the first, scientists connected insects, especially engraver beetles of the bark and wood of elm, with the Dutch elm disease. More and more, as the trouble was studied in different countries, these insects were held responsible for the spread of the *Graphium* fungus to healthy trees. We can disregard here, in the main, those other insects which develop in dead trees and those that, while injuring the living trees, have no part in the death of elms from this disease. Of all those mentioned, species of *Scolytus* have been referred to most frequently as connected with the spread of the disease. Spierenburg and Schwarz of Holland and Wollenweber of Germany were the botanists who first spoke of these insects as agents in the injury of the elms and as possible carriers of the *Graphium*.

¹ Rev. Appl. Myc. 11:156 and not his longer paper published in 1931 in Ann. Inst. Rech. Agron. Roumania, 3:240-256.

² Since the above was written the Dutch elm disease has been definitely reported from Spain (where previously its presence had been questioned) by José Benito Martínez in the following publication: *La Grafiosis del Olmo y la Demonstración de su Existencia en España*. Inst. For. Invest. y Exp. Madrid 9 no. 15: 1-29, 1936.

³ *Gartenwelt* 32:49-50. (Abstracted in Rev. Appl. Myc. 7:352.)

Spierenburg, as we have seen, mentions *Scolytus scolytus*. This was considered the chief carrier, but *Scolytus multistriatus* was added as a carrier in central Europe. Other early writers who considered Scolytus species in connection with the Dutch elm disease were Betrem (4, 5), Roepke (55), Grossman, Fransen and Buisman (22).

Later, A. Goidanich (23) claimed another species of Scolytus, *S. sulcifrons*, as the chief carrier in Italy. He also mentioned other beetles as possible carriers.

Finally Kalandra and Pfeffer (32, p. 16) stated that the known carriers of Graphium in CSR are *Scolytus multistriatus*, with its varieties *ulmi* and *triornatus*, *S. scolytus*, *S. pygmaeus*, *S. laevis*, *Pteleobius vittatus*, *P. Kraatzi*, *Magdalis armigera*, *Saperda punctata*, and in Bulgaria also *Scolytus sulcifrons*, and *S. affinis*. Some of these beetles develop two generations a year and so are more dangerous as carriers than others.

It was Fransen and Buisman (22) who made, so far as we have found, the most definite experiments to prove that *Scolytus scolytus* and *S. multistriatus* were the actual carriers of the fungus to healthy trees. In their experiments they placed these beetles in Petri dishes containing cultures of *Graphium ulmi* so that the insects became contaminated with the spores. Then, after a certain period, they placed 50 to 100 of the beetles within cheese-cloth coverings enclosing small branches of healthy elms. This was done in four periods from late May until after the middle of July and the beetles were kept enclosed for some time. At the same time similar check experiments were made with beetles not exposed to spore contamination, and other trees were directly inoculated with the fungus. After a limited period the number of living beetles, and the amount of chewing done by them was determined. Finally, in September and October, examinations were made of the health of the trees, sections taken of the branches for injury to the wood, and cultures made from the same for the presence of the fungus.

Although the results were not entirely uniform, in general they favored beetles as carriers of Graphium. Of the 88 trees in the experiments, 68 were finally used for the beetle tests. Of these, 36 were exposed to the fungus and 32 were not. Of the exposed number, 32 yielded cultures of *Graphium ulmi*, and of the unexposed, only 11 of the 32 trees contracted the disease.

The scientists also found that beetles placed on the trees early in the season caused a larger percentage of infection than those used in July, though the chewing may not have been any greater.

Measures Used in Controlling the Disease

In Europe a certain amount of work has been done in destroying dead and dying trees to limit the development of the fungus and of its carriers. However, no very strict measures have been enforced in an attempt to eradicate either. This was partly because the disease got so extended a start before the investigators determined its cause, and partly because there were no definite laws or authorities to put control measures into effect. It was also apparently a matter of opinion as to how practical such a method would be. Some investigators held that weather conditions had an influence on the injury and death of the trees; others held that the

fungus was not always fatal to the infected trees and kept them standing as long as there was a chance of survival. Lack of sufficient money for such a widespread and complete destruction of infected trees as eradication calls for was also a factor.

Although Holland, where the trouble started and has been most widespread, has a law (2) for the removal of infected trees, there is apparently no general belief that the fungus can be exterminated. Certain scientists in England believe that the trouble is not so fatal as to warrant complete destruction of all infected trees, or, at least, they consider the expense too great to try such a system. Likewise in all of the European countries the methods have been for partial control rather than eradication and even the control methods have been more or less haphazard.

We have cited tree injection as one unsuccessful attempt at control. Apparently spraying, while good for certain fungi and insects, has not been found worthwhile directly with this trouble. Where feasible for park and street trees, watering in dry weather has been advocated sometimes. Also in certain regions authorities have advised the barking of dead trees to prevent development of the fungus and the beetles, and also placing the logs in water for similar reasons. The chief method, however, has been the gradual removal of dead and dying trees.

Apparently the most intensive work for control has been the determination and planting of elms resistant to the disease. Holland, Germany and Italy lead in this field of investigation. The work has included the selection of resistant seedlings, the determination of such species and their varieties as are naturally resistant, and the grafting of the resistant species on roots of susceptible native varieties. For small trees the use of the resistant species *Ulmus pumila* and its variety *pinnato-ramosa* seems to have given promising results. Apparently the species of trees most resistant to the disease have come from eastern Asia, which might indicate that the disease originated there. In one case, at least, a seedling of a susceptible European species seems to have shown resistance.

During September, 1935, the senior writer attended the Sixth International Botanical Congress at Holland and had the privilege of seeing some of the work done by Doctor Buisman and others at Baarn. There visiting pathologists were able to see the inoculation experiments on different elms and a special exhibit of cut branches grouped according to their susceptibility or resistance to this disease. The species and varieties so displayed were as follows: Susceptible—*Ulmus japonica*, *U. hollandica*, *U. hollandica belgica*, *U. foliacea*, *U. glabra*, *U. americana*, *U. alata*, *U. racemosa*, *U. crassifolia*, *U. laevis*, *U. elliptica*; more or less resistant—*U. pumila*, *U. Wilsoniana*, *U. parvifolia*, *U. sieboldii*, *U. foliacea* (1 seedling), *U. glabra fastigiata*, *U. foliacea Wredei*, *U. foliacea Dampieri*, *U. hollandica vegela*.

HISTORY OF GRAPHIUM IN THE UNITED STATES

Warning. The danger of the Dutch elm disease being brought into the United States was called to the attention of our public in 1928 by Bartlett (106). He had recently returned from Europe where he had seen some of the damage wrought there. His publication included four of the early European articles issued by Schwarz¹, Wollenweber¹, Malcolm Wilson, and Malcolm and Mary Wilson. The next year Metcalf (158) called the attention of the tree workers at the National Shade Tree Conference to this same trouble and in June, 1930, Haskell and Wood (135) also warned against its importation.

Discovery. In an illustrated poster dated July 3, 1930, Charles F. Irish & Co. (137) reported this disease from Cleveland. Accompanying the poster was a short printed article from the Ohio Experiment Station stating that Doctor May had found diseased trees in both Cleveland and Cincinnati, and that Doctor Buisman of Holland had confirmed identification of the fungus. On August 8, 1930, an article by May (146) appeared in *Science*, and in the same year he (147) reported to the National Shade Tree Conference along the same line. May and Liming (156) also published a short account of this disease in an Ohio Experiment Station Bi-monthly Bulletin in 1931, and Liming (141) gave a further report to the Eighth Shade Tree Conference in 1932.

In the six intervening years since its discovery here, more articles have been printed in the United States than we have listed in the 15 years since the trouble has been known in Europe. Most of the former have been popular papers based largely on information already gained in Europe, or short articles recording some local data obtained in this country. Many of them appear in our bibliography from which we have gathered the following information.

History. Upon the discovery of the trouble in Ohio, the office of Forest Pathology of the United States Department of Agriculture co-operated with the Ohio Station in investigating the trouble. Liming (140) reported eight trees found there in 1930 and 1931 and these were destroyed. The next year no new infected trees were found, and it was thought that the trouble might have been exterminated. This seemed more likely since the European bark beetles of the genus *Scolytus*, as possible carriers, had not been found in that state. However, Beattie (110) reported another infected tree there in 1933 and two more (117) for 1934. We understand that a larger number was found in 1935 but so far none in 1936, so that one cannot yet be sure of the final situation in Ohio.

The disease was seen in New Jersey in September, 1932, by a park foreman, Richard Walter, in a Memorial Park at Maplewood, but specimens were not definitely identified by cultures until the spring of 1933. Its occurrence was published in *The Shade Tree* leaflet by Beattie (109) in July, 1933, and at that time he stated that 25 diseased trees had already been located in that vicinity.

Soon after its discovery in New Jersey the trouble was found in New York State both on the main land and on the islands in the vicinity of

¹ Articles by these authors translated by Mrs. Kelsey were included in the Bartlett collection.

New Jersey. Late in the fall of 1933, one infected tree was also found in Connecticut in the same general locality as the New York infections.

Stevens (168) in the *Plant Disease Reporter* of September 15, 1933, reported 388 infected trees in New Jersey and 14 in New York. Beattie (111) also reported for 1933 that diseased elms had been found as follows: New Jersey, 762; New York, 83; Connecticut, 1; Maryland, 1; Ohio, 1. Rankin (164) for 1934, reported 2,260 diseased elms had been located in New York State.

The tree reported by Beattie from Maryland was found in Baltimore in September. It was a short distance from a pier where imported elm logs had been unloaded. From these logs, according to Beattie (110), May and Fowler isolated *Graphium ulmi*. About this time Gravatt and Fowler (134) reported shipment of European logs to Norfolk, Virginia, and in some of these not only *Scolytus scolytus* and *S. multistriatus* were found, but *Graphium ulmi* cultures were obtained from both the beetles and the logs. These discoveries indicated the way by which the disease was brought into the United States. Soon afterwards, the United States Quarantine (170, 87) against the importation of elm logs from Europe was put into effect. In 1936 a few additional infected trees were found in Maryland.

Finally, in tracing the destination of imported logs in this country, Beattie (112) reported that in 1934 four infected trees were found in Indianapolis, Indiana, and one (113) in Norfolk, Virginia. In both cases these trees were not far from roads over which imported logs had been carted, and the ones in Indianapolis also grew near a veneer factory. At Indianapolis the infected wood in one of the trees went back to the 1926 ring. In 1936 at least one additional tree was found at Norfolk and several at Indianapolis.

So far, according to Beattie (113), the *Graphium* fungus has been isolated from logs at four ports of entry in the United States: New York, Baltimore, Norfolk and New Orleans. In the *Plant Disease Reporter* for February 15, 1935, the same author (116) gives a general account of the entry and movement of elm burl logs in the United States, as does also Worthley (183).

It has been the New Jersey-New York area that has caused the most concern because of the large number of infected trees found there. White (174) of New Jersey wrote in December, 1933: "A total of over 700 infected trees in this area (600 square miles) has been found." Dodd (124) in February, 1934, stated: "To date approximately 105,000 elms have been examined . . . and 3,200 suspected cases of the Dutch elm disease have been found," but in April of the same year he (125) further stated: "During the 1933 summer program, 740 diseased elms were found; 460 additional infected elms have been found during the winter months, making a total to April 19 of 1,200 diseased elms in New Jersey. With the exception of 100, all of these diseased elms have been removed and destroyed." Anon. (95) issued in *The Shade Tree* leaflet January, 1935, gives the number of diseased trees found in New Jersey from June, 1933, to February, 1935, as 5,133; all except 1,322 had been removed, and apparently the remaining trees were taken down later.

Filley (132) writing in February, 1936, concerning removal of trees both in New Jersey and New York, states: "In New Jersey and New York

about 6,300 trees were confirmed and removed as Graphium cases in comparison with over 7,600 in 1933 and 1934." May (152), who has had charge of the cultures made by the United States Department since the beginning, reports that in 1934 his department isolated the fungus from 6,913 trees, and from the beginning of the work in Ohio in 1930, until January, 1935, 7,790 cases of the disease had been confirmed. Most of these trees were in New Jersey and New York.

White (172-179), as shown by his writings, has been very active in the New Jersey campaign. He sent the original specimens from there to Ohio for final identification. The New Jersey Federation of Shade Tree Commissions, the State Department of Agriculture, and the State Experiment Station with the United States Department of Agriculture, have had to do with the campaign for and the removal of the infected trees in New Jersey.

As soon as it became apparent that this was the worst infected region in the United States, the Office of Forest Pathology removed its laboratory from Ohio to Morristown, New Jersey, and eventually established well-equipped laboratories for culture work, and greenhouses for experimental work with both the fungus and its insect carriers. Much of the investigation made there has not yet been published in detail.

Two circulars, one by May and Gravatt (154) published in May, 1931, and a second by May (150) in August, 1934, have dealt with the scientific side of the subject. A third circular by Worthley (181), who succeeded Kellerman (138-139) in charge of the eradication work by the Government, deals chiefly with this phase of the subject.

From time to time the government employees have also issued short notes dealing with certain phases of their work. We mention here one by Verrall and Graham (169) who found that the Graphium disease could be transmitted from one tree to another by natural root grafts; also one by Collins (122) who has worked with insects as carriers of the disease. The latter writes: "To summarize, two species of beetles, *Scolytus multistriatus* Marsh and *Hylurgopinus rufipes* Eich., have acted as carriers of the Dutch elm disease, *Ceratostomella ulmi*, in the laboratory experiments and have been closely identified with it in the field."

New York, when the disease was found there, quickly appropriated money for the destruction of infected trees. Rankin (163-164), under the State Department of Agriculture and Markets, has had charge of control work in coöperation with that carried on by the United States Government. New York, also, through Cornell, has provided for a laboratory for investigation of certain shade tree problems. One bulletin (171) has been issued by the Cornell Extension Service.

Besides the above persons in government and state service, several individuals have written on this disease for one reason or another. Among them are Felt (128-130) whose great interest in shade trees has led him to suggest methods of control; Reynolds (166) who has aroused interest in the subject in Massachusetts; Faull (126-127) who strongly believes that the disease can be eradicated in the United States; and Rex (165), of the New Jersey State Department of Agriculture, who has reported on eradication work in New Jersey.

Control Measures. Because few elms were involved in Ohio, eradication by cutting down all infected trees was necessarily attempted. When the trouble was found in New Jersey, the authorities tried the same control measure, and in the main, it is the method adopted by the United States Department of Agriculture under the various heads that have been in charge, now immediately under Worthley. The different states where the disease has been found have coöperated with the Government forces to obtain favorable results. We understand that at the end of 1935, practically all infected elm trees found in the United States up to that time had been removed, as well as many of the injured and dying trees not infected but in a condition to serve as breeding places for possible insect carriers.

A press service letter of the United States Department of Agriculture, issued under date March 9, 1936, and given out by Lee A. Strong of the Department, states: "The crews have marked for destruction 1,470,680 trees since the United States Department of Agriculture, in 1933, started its campaign to save the American elm from extinction. Of these 1,064,707 trees have been removed. Laboratory tests confirmed the presence of the infection in 14,233 trees¹. All but four of these have been destroyed according to the latest progress report of the Bureau of Entomology and Plant Quarantine."

At first merely infected trees, as found after laboratory identification of the disease, were destroyed. The eradication involved not only the destruction of all parts of the tree above ground, but also the digging out and burning of stumps. Later creosoting the barked stumps was tried and still later finely powdered copper sulphate was placed between the bark and wood to kill the fungus and prevent sprout growth. When the disease was found in the forests, especially in swamp areas, this method of eradication became very costly and cumbersome, especially so because of the rather insignificant value of the elm as a forest tree, and because in many cases these trees were doomed to die regardless of whether or not they had the disease².

As more was learned concerning insects as the chief or only carrying agents of the disease, it seemed important to limit their breeding. At first some few persons believed that the insects could be eradicated, but now the general opinion is to the contrary. This is largely due to the widespread distribution of *Scolytus multistriatus* in this part of the United States, and particularly because of the more abundant propagation of the native bark beetle, *Hylurgopinus rufipes*, which may become a considerable agent in the spread of the disease. These factors have led to the destruction of all dead elm trees and dead branches to limit the development of the beetles, and such wholesale methods have increased costs.

Whether or not *eradication* of the disease can be accomplished is open to question. Faull is most outstanding in his statements that this can be

¹ A still later report given in the *Plant Disease Reporter*, dated September 15, 1936, states that 20,808 trees had been confirmed as having the disease and 19,576 of these had been removed. Of these trees only 228 had been found in Connecticut, all but two of which had been removed.

² We understand that recently, as a measure of economy, the U. S. Government men are advocating the copper sulphate method of killing both healthy and diseased elms in certain swampy areas where the Dutch elm disease has become established or where it is likely to be established. The bark around the tree is girdled, and the copper sulphate dust is sifted on the wood. The girdled area is tightly bound by an oil cloth collar. This treatment is said to kill both the trunk and the roots and apparently exempts them from further or future attacks by the Graphium fungus or its insect carriers.

done and the government officials are certainly working on this supposition. The writers, however, are very doubtful that this can be accomplished. The 1936 season should determine whether it is desirable to spend further millions of dollars for eradication, or smaller amounts in useful control measures limited to streets, parks and private shade trees.

Coupled with eradication, which means a loss of every infected tree and many so-called half-dead trees that are not infected, have come other sanitary measures chiefly practiced in Connecticut (123), New York (171), and New Jersey (95, p. 3). These include removal of dead elms and dead branches, proper fertilization and watering of living trees where needed, and protection of their foliage by sprays against insects and fungi to promote the general health. While there has not yet been time to determine whether spraying will directly limit the spread of this disease, it will benefit most trees. It may also possibly lessen infection indirectly, since some writers believe that unfavorable environment has had something to do with spreading this disease.

Fate of our Elms. What, then, is to be the effect of this disease on the elms in the northeastern part of the United States? At the time of its discovery in New Jersey and New York, some people believed that, unless something were done to prevent its spread, in 10 to 15 years there would be no living elms in this part of the country. As measures have been taken, no one can prove or deny this hypothesis. Serious as has been the disease in Europe, no country has, as yet, lost all of its elms in this length of time, and most of these countries have done comparatively little to curb the ravages.

We are inclined to believe that the trouble will be less serious here than it has been abroad. In different parts of the same country where the disease has been found, there seem to be other factors than the fungus itself that have affected the elm trees. This is especially true in England. We are of the opinion that the insect carriers will be less common factors here in New England than they proved to be in central-western Europe following the World War, especially where gas injury to the trees apparently favored their multiplication. We also believe that the shade trees in New England have on the whole received better care than in many countries in Europe and that this will have its effect. Finally, we believe that the attempted eradication methods already used will result in benefit even if they are modified into more conservative control measures. We expect, therefore, that many of our elms will survive most of the investigations of their trouble. All of these, however, are merely personal opinions.

HISTORY OF GRAPHIUM IN CONNECTICUT

Field Work

Preliminary Search. In the year 1927, Dr. A. J. Wakeman of this Station complained of the yellowing and death of leaves (Plate I, 1) and the gradual death of twigs and smaller branches of two elm trees on his place in New Haven. As no external sign of a fungus showed on the leaves and twigs, cultures were made from discolored spots in the wood and these revealed a definite fungus as a probable cause. Likewise twigs were obtained in 1928 from trees at Greenwich by Marshall, of the United States Forest Laboratory at New Haven, and these showed similar injury and the same fungus was isolated from them. Some slight injury followed infection experiments with the fungus on seedling trees. Short notes on the trouble were made in the reports of the Director¹ of this Station and finally the fungus was identified as a species of *Cephalosporium* mentioned by May² and later given a more extended description by Goss and Frink.³

Our first work with this fungus suggested that it might be the Dutch elm disease, so we asked and received cultures of the latter from Doctor Westerdijk of Holland for comparison. While there were certain features in common, it was apparent that the fungi were distinct. This determination was made certain after Doctor Buisman visited our laboratory in 1930 and saw our cultures and the New Haven trees from which they came.

After the Dutch elm disease had been reported from Ohio, greater effort was made to watch the elm trees of our State for any indication of it. In the summer of 1933, upon the verification of the disease from New Jersey, we sent a written warning to the licensed tree men of the State requesting them to look for any suspicious injuries of the elms and to submit sample branches. The federal men (168), who made a survey of the Dutch elm disease in the eastern part of our country, also included the Connecticut elms in their examination. Our department, after making cultures from infected trees in New Jersey (Plate II, 2) and New York, made several trips to examine the elms in the southwestern part of the State near the New York line where the disease had recently been found. However, neither the government or state surveys revealed the presence of the trouble at this time.

Field Work by Years

Discovery in 1933. It was in the fall of 1933 that the first infected tree was found. This was on the Cushman estate at Glenville, near the highway, and close to the New York line. It was found by E. W. Dunbar,

¹ Bull. 322:119. January, 1931. Bull. 337:462. April, 1932.

² Nat. Shade Tree Conf. 7:91. 1931. *Science* n. s., 74:437. October, 1931. U. S. Plant Dis. Rptr., 15:143-144. November 1, 1931.

³ Neb. Agr. Exp. Sta. Res. Bull. 70:1-24. f. 1-14. January, 1934.

formerly of the Bartlett Tree Co., who had worked previously on this particular tree. Specimens were sent to the government laboratory and the *Graphium* fungus was isolated. Later, on October 12, we visited the tree (Plate III, 1) and found it nearly dead with few adhering leaves. Our laboratory also isolated the fungus.

Other sickly trees at this same place and nearby failed to yield the fungus. We also saw the tree when it was taken down and burned on January 16, 1934. While the limbs were quite brittle and broke when falling to the ground, we saw no sign of the *Graphium* fruiting stage on any of them or on the main trunk, so it was not much of a menace to the surrounding trees. The death of the tree, too, could not be laid entirely to the *Graphium* fungus, since a Basidiomycete, *Armillaria mellea*, was responsible for a general rot of the bark and wood on the main trunk.

Work in 1934. With the finding of this tree, the United States Government placed L. R. Fate directly in charge of the situation in Connecticut. At first his headquarters were at Greenwich, later at Stamford, and he continued the removal and destruction of infected trees in this State under the Station's approval up to the late spring of 1936 when he was succeeded by T. H. Cannon. Fate's men began making a survey by inspecting all the dormant elm trees in this vicinity. They found several more diseased trees in Greenwich before the trees began to leaf out in the spring of 1934. The second case was an elm growing in the backyard of T. B. Lyon at Greenwich in February. Plate III, 3 shows the destruction of this tree. The fruiting stage of *Graphium* appeared somewhat in the galleries of *Scolytus multistriatus* in the dead and dying bark.

Before the advent of the growing season of 1934, Director Slate of this Station called a meeting of the Botanical, Forestry and Entomological departments to devise means for the study and control of Dutch elm disease. The Botanical Department was assigned the identification and study of the fungus, the Forestry Department the control work, and the Entomological Department the study of the insects involved as carriers. The State was divided into four general areas as follows: The infested area of Fairfield County was left in the hands of the federal men. Then the remainder of the State was divided into three sections as follows: (1) Reaching from the Naugatuck river and a line to the north to include the territory to the western boundary of the State; (2) from the Connecticut River westward to this limit; (3) from the eastern end of the State westward to the Connecticut River. Forester Filley had charge of the scouting of these areas and Dr. L. B. Arrington of Massachusetts was appointed the general supervisor for coöperation with the Botanical and Forestry departments.

At the end of the year the federal men reported 55 infected trees in their area as follows: Greenwich, 35; Stamford, 9; Darien, 8; Norwalk, 1; Fairfield, 2. Our laboratory made cultures from certain of these infected trees, verifying one or more in each town except Fairfield. In this case we failed to get *Graphium* from the two young trees reported by the Government.

We obtained only *Cephalosporium* sp. in the several cultures made. No further *Graphium* trees have been found in this locality and each year we have obtained only the same *Cephalosporium* sp. in the trees tested there. Our cultures for the year totaled over one-third of the infected trees found. Not much chance was left to study the effect of the fungus on the health of the trees as most of them were cut down soon after they were found diseased. Those examined showed little or no fruiting stage of the *Graphium* so the chance of spreading the disease was not great. We saw one big tree from which a large branch had been removed because of wilting. At the time we looked at it, there was a slight wilting and yellowing of two or three small branches, but we learned later that the tree had to be cut down because of general infection that followed. Evidently cutting off the large limb had not removed all of the infection and serious injury to the rest of the tree followed rapidly.

Our department made many tests of twigs collected in the sections of the State surveyed by the Station but only one tree gave cultures of *Graphium*. This was at Old Lyme and made the fifty-sixth infected tree found in the State that year. Specimen twigs were gathered by one of Mr. Filley's men on September 6, and cultures of *Graphium* were obtained on September 10. We examined the tree on September 11 and several more times before it was finally cut down and destroyed. It grew in a private lane (Plate III, 2) and was one of an avenue of large trees several of which looked just as unhealthy and had shown trouble for a year or two. However, neither we nor the Government obtained *Graphium* cultures from any except this one. When cut down, the diseased tree showed the *Graphium* fruiting stage in great abundance in the channels of the bark and wood made by the native elm bark beetle *Hylurgopinus rufipes*¹ (Plate IV, 2). As the European bark beetle, *Scolytus multistriatus*, according to our entomologists, had not been found in this vicinity, it was questioned how the tree became infected unless by the native beetle.

This brought up the problem of insect carriers in the State. The entomologists state that *Scolytus multistriatus* occurs more or less commonly in the infested regions in Fairfield County, and also outside of this area, but apparently not much east of the Connecticut River. So we took up the possibility of infection by *Hylurgopinus rufipes* which occurs over the State and was not uncommon in Old Lyme. We obtained beetles from the channels where the fruiting stage of the fungus was found and others from the twigs where it was not evident. These were placed in sterile Petri dishes containing nutrient agar, and growths of *Graphium ulmi* developed. Likewise we obtained similar growths from their larvae and from mites and ants taken from these fruiting channels. Native beetles from channels of other elms, a few miles away and not infected, failed to develop such cultures. Next we tried to get cultures on sterilized healthy elm twigs by placing them in test tubes with beetles from the infected tree. After the beetles chewed into the bark, we succeeded in most cases in getting the coremia of this *Graphium* on the twig.

This experiment showed that the native elm bark beetle was a possible carrier and it was left to the entomologists to study its life history and

¹ Station Bulletin 387 takes up the morphology of these beetles.

especially its feeding methods on the young twigs of elms in the spring. There is little doubt in our mind that if the mature beetles carry the fungus in the fall from the infected bark to the dead bark of an uninfected tree *Graphium* will develop in the latter. This would increase the possible sources of contagion. Our results were published in *Science* (121).

Middleton (159) about this time wrote concerning the engraver beetles as follows: "There is likewise an American species of engraver bark beetle, *Hylurgopinus rufipes* (Eich.), infesting elms in the infected region. Experimental proof that the engraver bark beetles inoculate elms with the Dutch elm disease in this country is still wanting." Shortly afterward McDaniel (157) of Michigan wrote of *Hylurgopinus rufipes* in these words: "It has, during the season of 1934, been the immediate cause of death to many trees which might otherwise have survived. The prolonged drought, together with the lowering of the water table, has so weakened elms in this section of the country that this species of bark beetle has become unduly prevalent. Though this beetle has not as yet been known to carry the Dutch elm disease, its habits are such that it should come under suspicion in sections where the disease is discovered." We believe our article was the first direct evidence of this nature.

After finding the isolated tree at Old Lyme, we examined all the dead and dying elms in the State on which our entomologists had found the native and foreign engraver beetles at work. Our object was to see if the *Graphium* fungus could be found in their channels or in the adjacent dead and living bark. These trees, including those we found ourselves, numbered about 100. Many of them were in the infected area of Fairfield County and were pointed out by Fate. These latter trees were mostly still living and not so far advanced as to show the *Graphium* fruiting stage, if present, in their bark.

In the remainder of the State, where the disease had not yet been found, we were unsuccessful in finding the *Graphium* stage of this fungus though in many cases the bark was in a proper condition to show it if it were present. On one or two trees we did find, in small amount, a *Graphium* that was evidently a different and apparently a saprophytic species. These observations confirmed our opinion that *Graphium ulmi* is not a native species in this State. If we find it in these isolated regions scattered over the State at a later date, we shall know it has been carried there.

One can distinguish the native beetle, *Hylurgopinus rufipes*, from the imported species not only by its dull appearance but also by the tunnels it makes between the bark and the wood. These are more or less horizontal (Plate IV, 2) while the tunnels of the shiny *Scolytus multistriatus* are usually vertical (Plate IV, 1).

Work in 1935. While we made a number of trips over the State and looked for suspicious elm troubles during the year, we depended mostly on Mr. Filley's men for scouting work. We did examine the most suspicious trees that they found in an effort to get further data. Most of these, however, failed to show the *Graphium* fungus. We obtained data and cultures from two young trees, discovered by the Government men at Darien (Plate II, 1). The disease appeared generally over the trees and seemed to have developed rather quickly.

At Old Lyme four more live elms showed the trouble and cultures and data were obtained from these before they were removed. Otherwise all of the new infections were found in Fairfield County and totaled 76. Only one new town Westport, was added to the previous list.

The Government's report of infected trees by towns for 1935 was as follows: Westport, 1; Norwalk, 4; Old Lyme, 4; Darien, 6; Stamford, 27; Greenwich, 34. The number of infected trees found in Connecticut (132) before January 1, 1936, totaled 133. All of these, as well as many dead and dying elms that were not infected but were the possible breeding places of beetle carriers, were removed under the direction of Fate and Filley.

Work in 1936. Since the State hired no scouts under Filley this year, fewer cultures were made from suspicious elm trees than during the previous year. Such specimens as were tested were collected by the writers, Mr. Filley's assistant, Philip P. Wallace, and correspondents who sent in specimens chiefly from uninfected districts scattered over the State. On the other hand the numerous Government agents collecting in the infected districts reported the largest number of infected trees for any year so far, namely 102 up to the middle of October. These infected trees, as reported to Mr. Filley, were as follows: Branford, 1; Guilford, 1; Ridgefield, 1; New Canaan, 5; Old Lyme, 1; Westport, 3; Norwalk, 7; Darien, 9; Stamford, 11; Greenwich, 63. The disease was new in the first four towns listed.

The six trees reported from Ridgefield and New Canaan were cut down before we had a chance to confirm the Government tests, as we aim to do when the disease is found in a town not before reported. The tree at Branford failed to yield the *Graphium* when cultured by us and in the second test made by the Government just before the tree was cut down. We did get *Cephalosporium* sp. from this tree and from an adjacent tree also cut down. Tests were made from two other near by trees but these also failed to show *Graphium*. This failure makes the infected tree somewhat questionable to us as was the case of the two trees reported by the Government in 1934 from Fairfield. However, it is quite possible that the Government men in their tests got the only *Graphium* infected twigs. We did, however, confirm the additional tree found at Old Lyme and also the recently reported infected tree (October 8) at Guilford.

Total Infected Trees. The number of trees found infected with *Graphium ulmi* in Connecticut up to October 10, 1936, were as follows: 1933, 1; 1934, 56; 1935, 76; 1936, 102. This makes a total of 235, counting the three unconfirmed trees found in Fairfield and Branford where no further infected trees have been reported. It will be seen from this data that the diseased trees found each year have increased in number. While these infected trees are few as compared with those found in New Jersey and New York the results, after four years of search for them, point toward control rather than eradication.

Appearance of Diseased Trees in Nature

General Statements. Our experience in following the Dutch elm disease, from the first signs of infection on a tree until final death, is somewhat limited. In the first place the disease has been known a comparatively

short time in Connecticut. Secondly, the authorities have acted promptly in taking down infected trees, so that no one has a very definite idea of what might have happened if these trees had been left to fight the trouble naturally. However, we have seen infected elms of different types in New Jersey, New York, Connecticut, and Holland (Plate II, 3) and a few suspicious ones in England, France and Germany. We agree that there are two types: The first destroys the trees quickly, apparently one or two years after infection; the second acts slowly so that trees lag along with dying branches for a number of years. We have seen both of these types in Connecticut and elsewhere.

We are not certain just what makes the difference in this rate of the disease. We suspect, however, that it depends on how fast the trouble reaches and kills the cambium layer. Where the disease is confined to isolated spots in the wood-rings, and where no or little injury shows in the cambium, we believe that slow death, or possibly apparent recovery of the tree, as has been mentioned in England, occurs. But once the disease gets a good foothold in the cambium, injuring it in spots or with a complete blackened ring, there results a general death of the branches all over the tree and rather sudden demise. A single local or many natural inoculations over the tree might account for these differences.

We are not sure what causes the death of the tissues. So far as we have seen, there is no conspicuous amount of mycelium of the fungus killing them by stealing the food necessary for growth of the tree. The injury seems more likely to be due to the toxic effect of this fungus indirectly killing the tissues. This injury also brings up the question of death because the water-carrying tissues are clogged, depriving the leaves of nourishment so that they wilt and die. We have not seen much of this clogging in the trees examined. We believe it is chiefly found in old, dead trees where bacteria, etc., have come in subsequently, as is mentioned in European accounts. It seems likely that the fungus is never very abundant in the tissues but that its toxins might cut off the water supply to the leaves by directly killing the water-conducting tissues in the wood or, if reaching the leaves, by killing the tissues there.

Effect on Leaves. In any case the first effect on the infected tree is injury to its leaves. This usually shows as a wilting and then a curling and drying up, often while still greenish (Plate I, 2). In some cases the leaves turn yellow, or dry up and become brownish. Usually they do not adhere long, though the few immature terminal leaves may cling to a surviving branchlet after the older leaves fall off. Such branches are said to develop a more or less wilted crook to their tips. Often a single branch or twig, showing such wilted leaves when the rest of the tree looks normal, may be the first sign of infection. As we have previously stated, in severe cases there is indication of such leaf injury generally over the tree. Sometimes where the tree has been pruned, the adventitious twigs also show the leaf injury.

One, however, must learn to distinguish this trouble from leaves injured by insects, sprays or storms, especially from wind storms along the sea coast which often occur in late summer and autumn. Likewise, broken branches, which still adhere to the tree, may fool the careless observer. Leaf injury is best looked for in this region from the middle of June until the last of August.

We have never seen the coremia of this fungus on the leaves or petioles of infected trees, although we have kept leaves and small twigs for some time under a moist condition in bell jars to see if they would develop. However, we have never seriously attempted to recover the fungus directly from the petiole, midrib or other tissue of naturally infected leaves. May (150, p. 11) reports that he has recovered the fungus from leaves of artificially inoculated twigs. The evidence on the whole, however, seems to be doubtful if infection naturally occurs through the leaves.

Effect on the Twigs. After finding suspicious symptoms of injured leaves, the next step is to study the twigs to which they are attached. This is done by cutting across twigs a quarter to half an inch in diameter. If discolored spots (Plate V, 1) are found in the wood (usually here in Connecticut in the growth rings of this, or the previous season) the trouble is more likely to be of this nature. Cutting the twig lengthwise will show the injury as a line of darkened tissue. If this browning is very evident in these cross or longitudinal sections, it indicates great injury, especially if the cambium is invaded. By stripping off the bark to the white exposed wood, the injury to the cambium appears as brownish streaks, or a general invasion which in cross-section shows as a circle of discolored tissue. The presence of a fruiting stage, as external coremia in the young branchlets attached to the tree, or after they have fallen to the ground, evidently is not common here and so far has never been seen by us.

But none of these symptoms are positive indication of the Dutch elm disease, as suggested in our earlier statement concerning the *Cephalosporium* disease. Investigations of workers in all of the countries where the Dutch elm disease has been found have revealed that other fungi produce spots and injuries in the rings of the wood that are difficult to distinguish from the work of the *Graphium* fungus. Those that we have found have yielded cultures not only of *Cephalosporium*, our most common elm twig trouble, but of *Verticillium*, *Sphaeropsis*, mixed fungous and bacterial growths, and other injuries that give no growths at all. These troubles will be mentioned briefly later. With these injuries to the wood by other fungi, it becomes necessary to obtain a growth of the *Graphium* fungus before one can be sure that the trouble in any tree is due to the Dutch elm disease. So far we have not been able to confirm the disease absolutely without such evidence. If the twig comes from a district that is known to be infected, one can usually make a correct guess, particularly if he has seen the tree. On the other hand, he will sometimes fail in his diagnosis on a twig that has all of the symptoms but comes from an uninfected district.

General Effect on Trees. We have shown that large limbs and finally whole trees may become infected and eventually die. But there is no way of distinguishing this trouble from others by merely looking at a tree. Dead and dying branches result also from winter or drought injury or from certain other fungi like *Armillaria mellea* or *Collybia vellutipes*. These fungi, however, can usually be detected by the presence of their fruiting stage, as toadstools, in the dead bark of the main trunk or large branches. If the infected branches are entirely dead, the spotting by the *Graphium* is usually not so evident as it is on the living ones. When the disease is

well advanced on old but still living trees, one can often detect the disease in the cambium by peeling the bark away from the wood and tracing the brown streaks from the infected branches more or less continuously down to the base of the tree.

This was the case in one of the trees found at Old Lyme in 1935. Not all of the branches were infected, but those that showed the dead leaves revealed these streaks in their smaller branches, and from these ran into the main trunk where we traced the streaks down into the roots of the tree on that side. Furthermore, we recovered the *Graphium* fungus from these small infected branches, from the main trunk and from the roots where these lines occurred. This tree and its infected branches were not far gone when cut down and so there was no growth of the fruiting stage in its bark. If it had been left standing it might have lived for some years, and when death occurred we have little doubt that an abundance of the *Graphium* fruiting stage would have been found in its bark.

Inoculation Experiments. Unfortunately we have made no inoculation experiments but have had to depend on results published by others. This work has been done in the past chiefly by Schwarz and Buisman of Holland, Wollenweber of Germany and May of the United States. The reason we have not undertaken such work is chiefly because of the effort being made to exterminate the trouble here, but partly because the Station at New Haven is outside of the infected zone and we do not wish to be accused of possibly spreading the trouble. Greenhouse inoculations might be tried, but we have no facilities for extended work along this line, especially since great caution would be necessary under our conditions to prevent possible escape. We believe, too, that out-door inoculations under more natural conditions should be made on both small and large trees and that these should be watched from the beginning of the trouble until final death.

Inoculation of trees should be tried under different environmental conditions, such as low temperature, and low and high water supply to the roots in amounts ranging from insufficient to excessive. Further, different strains of this fungus should be used to see if any one, or a combination of them, affects inoculated trees differently. The number and places of inoculations, too, might be further extended as well as the time of year for such.

Cultures and Microscopic Study of the Fungus

General Statements. In 1933, in anticipation of a possible outbreak of the Dutch elm disease in Connecticut, a survey of the elm situation was made and sixty trees were cultured. From these nine *Graphium ulmi* cultures were obtained. Eight were from trees outside of the State and one was from the first infection found in Connecticut at Glenville. All nine trees had been previously diagnosed by federal workers, but they were recultured in this laboratory in order that we might become familiar with the best method of handling the fungus.

In 1934 twenty-one *Graphium ulmi* cultures were obtained from different trees. Twenty were duplicates of positive results already reported by the federal office, and one was a new infection at Black Hall, Old Lyme, discovered late in the season by this Station. During the summer, the Station had a corps of scouts who worked in parts of the State remote from the known center of infection at Stamford and who collected only material with very definitely suspicious streaks. The infection at Black Hall was the only one found by them.

In 1935 six new cultures of *Graphium ulmi* were isolated, of which five were from trees already condemned and one was from material collected by the senior writer at Amsterdam, Holland, for further comparison with our cultures.

During the present year, up to October, 1936, only three *Graphium ulmi* cultures have been isolated. One was from a tree at Old Lyme, which had been sampled but not yet reported by the laboratory at Morristown when our culture was made; the second was a verification requested by the owner of a tree in New York; and the third was the Guilford tree, already mentioned. Owing to low funds and to the fact that the infected areas were so well covered by federal scouts and that nothing suspicious had been found outside these areas in the two preceding summers, no scouts were employed by this Station in this current summer.

Most specimens cultured were collected by regular employees of the Station or were sent in by outsiders. In the spring and summer of 1935 the Southern New England Telephone Company coöperated with the Station by having their field men send in any suspicious specimens which they came across in their work. No *Graphium ulmi* was found in their samples.

Altogether there have been cultured 678 elm trees from which there were isolated 39 *Graphium ulmi* cultures, 239 *Cephalosporium*, 41 *Verticillium*, 7 *Sphaeropsis*, 10 *Fusarium*, 4 *Basidiomycete*. The remaining cultures were either sterile or developed indefinite mixtures.

After the infection was found at Old Lyme, that territory was taken over by federal employees who scouted there as well as in the Stamford region which had previously been assigned to them. The Station scouts made no inspections in the Stamford area and none in the Old Lyme vicinity after the first infection was found. This accounts for the low percentage of *Graphium ulmi* cultures isolated in this laboratory from Connecticut trees.

Microscopic Appearance of Infected Wood. The difficulty, if not failure, of detecting the fungus in sections and the abundance of tyloses in the diseased vessels have been noted by many workers, such

as Schwarz (57), Pape (41), Wollenweber (74), Buisman (10), Lendner (34) and Tubeuf (67). In literature probably the best known figures are those given by Wollenweber (77) and Buisman (67) showing cross sections of diseased vessels. More recently Banfield and Smith¹ state that spores, distributed by the cell sap, and a sparse mycelium are found in the discolored vessels, and that the spores bud yeast-like or form new hyphae which penetrate the bordered pits into adjoining vessels and there form new conidia.

In the present study the same difficulty in differentiating the fungus was experienced. This held true regardless of the plane of the sections, the thinness of the sections and the stains used. Transverse, radial and tangential sections were cut and stained with the Pianese stain, cotton blue, eosin, and diamond fuchsin with gentian violet and gold orange. The first sections were made from dried twigs collected in 1934 at Greenwich, Stamford, Darien and Old Lyme. On the whole the highly infected tree at Old Lyme showed the fungus more clearly than the trees from the other places. An unusual amount of the fungus was found in a streak in the main trunk of this tree (Plate V, 8). While cultures were not made of this particular streak, the general appearance of the fungus and its tendency to adhere closely to the walls and to become extremely attenuated where passage is made through the pits give it the same characteristics as the fragments of *Graphium ulmi* found in cultured streaks in twigs from the same tree as well as in those from other trees. At the point of penetration through a pit, the fungus seems to flatten out into a disk-like shape.

Sections were made from twigs from a tree infected with *Graphium ulmi* found at Old Lyme in June, 1936. An alcoholic solution of eosin was the stain most commonly used for these sections. Except in scattered places the fungus was sparse (Plate V, 5, 7). Here and there spores were found in vessels but they were not so abundant as one might expect from the results reported by Banfield and Smith. Possibly the degree of infection and the amount of water supply in the tree may be factors which largely control the behavior of the fungus in the wood. As reported by Banfield and Smith, there were hyphae penetrating the pits in the walls of the vessels. These hyphae may be so minute that apparently they pass through without any constriction; but, if larger, they become extremely attenuated at the point of penetration as noted in the material collected in 1934. There seems to be a general tendency for the fungus to adhere closely to the walls of the vessels, only occasionally extending into the lumen. Since the fungus is so readily isolated, it must be conceded that it is universally present in the streaks. This study indicates that there may be extremely attenuated hyphae in the wood and in such cases it is often difficult to detect the fungus in sections. In the material studied in this laboratory, conidia have not been found frequently enough to justify the statement that they are a common means by which the fungus is spread in the wood. No doubt, however, they are a contributing factor, and under certain conditions may be very abundant.

We have noted that the fungus may greatly increase in the vessels after the infected piece has been in a culture medium for a time. A small part cut from a twig of the tree at Old Lyme, 1936 collection, was used for

¹ *Phytoph.* 26:86. F. 1936.

immediate sectioning. The remainder of the twig was flooded with alcohol, flamed and debarked, and short lengths were cut with sterilized pruning shears into a Petri dish containing malt agar. At intervals up to 46 hours, sections were made of the cultured pieces and compared with sections made from the piece not cultured. Sections made from the piece not cultured showed a few scattered spores and a scant mycelium, as previously found in sections made from other twigs from the same tree. One vessel in a piece of the twig kept in the agar 46 hours, but not yet showing growth externally, had become nearly filled with conidia, while other vessels in the same piece of wood had few spores but a considerable amount of mycelium (Plate V, 6). No sections of the uncultured piece, nor sections made from other twigs from the same tree, showed so many spores or so much mycelium. In these sections, also, no fungus was found in many of the discolored vessels which may indicate that a toxin may extend beyond the actual presence of the scanty but very virulent fungus.

Cultures of the Fungus. In making cultures, twigs were flooded with alcohol and flamed. The bark was peeled back with a sterilized scalpel and pieces of the streaks (Plate V, 1) were planted on the agar. The agar media most commonly used were made with oats and with malted barley, both of which nutrients were ground at this Station and which proved very satisfactory. Plugs of potato and of carrot, grains of wheat, oats and barley, sterilized liver, elm bark and wood were also used. A solution of Taka-diastrase was added to some of the media, resulting in little change in the amount of growth. On the liver, small, compact, yellowish colonies of spores with little mycelium developed. In an acid medium, growth is somewhat retarded. Transplants were made from Petri dishes in which *Graphium ulmi* only had developed, but one-spore cultures were made for comparison with those transplanted in mass.

Mycelium. A culture of *Graphium ulmi* is characterized by short tufts of hyphae and sharply defined concentric rings (Plate V, 2). In older cultures fan-shaped zones may radiate from the center. At first the fungus is white but later it may become yellowish and covered with a bacterium-like ooze of conidia. Occasionally on agar there are scattered black areas where coremia or the staghorn growths are present.

The hyphae are straight or slightly wavy with parallel walls, though rarely somewhat bulbous cells may be found. Here and there ladder-like connections of the hyphae may occur (Plate V, 3). The largest hypha measured was 7.4μ in diameter, but that size is unusual, the average of those in our cultures being 4 to 6μ . In infected wood, hyphae are often less than 1μ in diameter, which is smaller than the smallest hyphae noted in cultures. Sharply defined septae form cells ranging from 5.5 to 35μ in length, and in each cell there are two to several glistening bodies. The branching varies from acute to right-angled.

Spores, Coremia, Sclerotia, etc. The mycelial spore formation, as designated by Dr. Buisman, or the so-called Cephalosporium type of spore, consists of short branches which in turn may be unbranched or have two or more smaller branches each of which has at its tip a cluster of hyaline, somewhat elliptical-shaped conidia (Plate V, 4). The conidia may be partially flattened lengthwise due, probably, to contact with each other

on the conidiophore. The conidia readily separate from the conidiophores, and may bud yeast-like, thus greatly increasing the spore output and giving rise in older cultures to the yellowish ooze mentioned above. They may also form germ tubes which very quickly develop conidiophores with spores. In studying the germination of spores, they were planted in a van Tieghem cell made with a loop of agar on the cover glass. As conidia continue to increase in size after separation from the conidiophore and buds of various sizes may be separated from the parent conidium, measurements were made of the largest conidia still attached to the conidiophore. The average size of such spores in our culture media was about 5.8 by 2.4 μ . Spores free from the conidiophore measured from 4.7 to 10 μ by 2-3.5 μ .

Coremia are not commonly found in agar cultures and when they occur in any abundance they are usually in freshly isolated ones. However they develop abundantly and consistently on elm twigs in test tubes and Petri dishes. In natural infections they are sometimes found on bark which is somewhat loose from the wood and beetle tunnels are favorable for their development (Plate IV, 3-5). While still retaining the characteristic brush-like top (Plate VI, 3), coremia vary considerably in size. The ones most familiar are very conspicuous. They have an erect, dark, only occasionally branched, solid stalk (Plate VI, 2) and a colorless, flaring head to which the spores adhere, forming a glistening, whitish, at first, and later slightly yellowish, droplet (Plate VI, 1). Measurements were not made of coremia which were found growing naturally on bark but undoubtedly they are much longer than those produced in our cultures in which they measured only up to 500 μ in length. There are also coremia with much shorter stalks which may be readily overlooked when a hand lens is used (Plate VI, 4). These may have relatively thick stalks, measuring about 51.8 μ , or they may be more slender, ranging down to a stalk of only one hypha, which nevertheless has a typically branched head of a coremium. The smaller and more slender ones frequently grow in tufts and their stalks may vary in color from light to dark brown. The coremial spores, which are smaller and more uniform in size than the mycelial spores, are hyaline, chiefly elliptical, and are borne on the brush-like tips of the coremia (Plate VI, 5). Spores of the coremia may be readily confused with the mycelial spores which are produced so abundantly on the straggling hyphae surrounding the coremia. For this reason measurements were made of the largest spores still attached to the coremium. The average size of 10 spores was 4.8 by 2.1 μ .

Germination of the spores of the coremium does not seem to be so high as that of the mycelial spores. Forty-eight van Tieghem mounts were made, each from a different coremium. A film of malt agar was put on a sterilized cover glass and spores of the coremium were obtained by touching the tip with a sterilized needle. This precaution was necessary to avoid mixing the mycelial spores with those of the coremia. Of the 48 mounts, 21 gave no germination, 9 only slight, and 18 abundant. Germination of the coremial spores is similar to that of the *Cephalosporium* type, forming a mycelium with secondary spores (Plate VI, 6). So far as we noted, yeast-like budding was restricted to the secondary spores.

Staghorn growths are at times very conspicuous in agar cultures (Plate VI, 7). They vary greatly in color, size and shape. They may be entirely

colorless or have a decided yellow tinge and they may be entirely black or have definite black and white areas which often form distinct bands. They may be simple or extensively branched. In cross-section they may be round or flattened and the tips may be greatly attenuated or broadly fan-shaped. They are at times short tufts, but they may also be considerably longer than any coremium found in culture. One measured about 12 mm. long. The factors which may lead to these grotesque forms were not studied. One culture, out of several made at the same time, on the same medium and kept under the same conditions, may produce them and the others may be free of them.

Small, round sclerotia, measuring about 40 μ in diameter, are found in agar cultures as well as on wood (Plate VI, 8). They are solidly built with a few layers of thick-walled, brown cells on the outside, and thin-walled, colorless cells within (Plate VI, 9). They may be very abundant or somewhat rare in cultures. Here also the factors which cause them to be produced in varying amounts, as well as their function, have not been determined. It is possible that they become a dormant stage of the fungus, though it has also been suggested that they may be undeveloped perithecia.

Dr. Buisman (13) found in *Ceratostomella fagi* small, imperfectly formed perithecia along with sclerotia. In *Graphium ulmi*, also, there may be found in single strain cultures small, imperfectly developed perithecia (Plate VI, 10), similar to those described by her. They have short, stout necks and apparently never form asci. Such imperfect perithecia were found in 17 different isolations made in this laboratory, but were only seen when the cultures had been recently isolated.

Perithecia. The cultures for studying the perithecia were obtained in February, 1935, from the Willie Commelin Laboratory at Baarn, where Dr. Buisman first reported *Ceratostomella ulmi* as the perfect stage of *Graphium ulmi*. The two separated strains were on cherry agar, and the two strains growing together were on a piece of sterilized elm twig. After receiving the cultures, perithecia continued to develop on the twig. Since receiving these two strains they have been repeatedly crossed with each other on elm twigs as well as on agar. A few agar cultures have produced perithecia in numbers sufficient for embedding in paraffin but on the whole they do not develop so quickly or so frequently as on elm twigs. A piece of elm twig sterilized with the agar seems to aid in their production on this medium. The advantage of the agar over the elm twig is, of course, the greater ease in sectioning in paraffin. On agar it is usually a month before perithecia appear. Even on elm twigs, however, there is considerable variation in time before perithecia become apparent. Some appeared four days after the culture was made, but usually it takes from one to three weeks. On elm twigs, as on agar cultures, there is a difference in the number of perithecia produced, even though the cultures have been made at the same time, and apparently made and kept under the same conditions. No record was kept of the number of cultures made with the two Holland strains growing together, but probably in more than half no perithecia appeared, in some only a few, while in others they were abundant.

Forty-eight crosses were made with cultures isolated from material collected in the United States, but no perithecia developed. Five cultures, isolated from two trees at Old Lyme, one from a tree at Greenwich,

one from East Greenwich and one from Darien, have produced perithecia when grown with one of the strains received from Holland. These five cultures, when grown with the other Holland strain or with cultures isolated from native material, in no case produced perithecia. The culture isolated from the material which the senior writer collected in Holland also produced perithecia when grown with one of the strains received from Holland.

The perithecia (Plate VII, 1), which have been repeatedly described since Dr. Buisman's discovery, are round and black with a few hyphal strands extending from the outer layer of the perithecial coat and the long neck, measuring 267 to 450 μ , is terminated by a fringe of colorless cilia (Plate VII, 3). Perithecia grow singly or in groups (Plate VIII, 8). Ordinarily they have only one neck but some have been found with a brown-celled partition in the perithecial cavity and each division has a neck, giving the appearance of two perithecia having grown together. In other cases there is only a thin-walled partition, each division having a neck, and again there is no partition at all in the perithecial cavity though the perithecium has two necks. In one case three necks were noted. The bulbous base measures 100 to 133 μ in diameter.

For paraffin sections the material was killed in Gilson's solution, Craf's solution, picric acid in 70 per cent alcohol, Flemming's solution and Benda's solution. The sections were stained with iron-alum haematoxylin, diamond fuchsin with lichtgrün and diamond fuchsin with gentian violet and gold orange. This study of the perithecium is far from being complete and no attempt was made to study the nuclear behavior.

The neck of the perithecium very early begins to elongate (Plate VII, 5-6) thus distinguishing it from a sclerotium. The coat usually consists of three layers of thick, brown-walled cells enclosing in the beginning a compact mass of thin-walled hyaline cells. There is a rapid differentiation of ascogenous tissue accompanying the elongation of the neck. In these sections there has been found repeatedly a trichogyne-like body which occupies the canal of the neck and looks like a hypha (Plate VIII, 7-8). It is enlarged in the perithecial cavity. In some cases it almost bears the appearance of having grown downward into the perithecial cavity, while in others it seems to be an extension of ascogenous tissue. It has not been definitely determined whether or not it is a stage in the development of a peculiar round or elongated body, which is frequently found in the perithecial cavity before the asci are mature and which is apparent until the ascospores are ready to discharge (Plate VIII, 1-6). This body is found in the perithecia which develop from the crossing of the two strains received from Holland as well as in the crosses made with one of the Holland strains and native material. It is found in perithecia grown on agar, as well as on wood, and treated with the various killing agents and stains mentioned above. The possibility that this body is connected with sexuality seems to be lessened by the fact that it is highly organized after the spores have begun to mature (Plate VIII, 4). If it is a parasite of a Synchronium-like nature, one might expect to find different stages in its development and a more highly differentiated final stage. Moreover one might expect to find some evidence in the culture, though outside of the perithecium it may be so small as to escape detection. There is also the possibility that it may be a food body but, although it stains black with

osmic acid, it seems too highly organized and varied in form for that. There is also the suggestion that it may be an aid in the exudation of spores from the perithecial cavity. Possibly with more material to section there may be found other stages which may throw some light on its nature.

The asci are slightly oblong when separated from each other (Plate VII, 8). This separation occurs before the ascospores are mature. Each ascus contains eight colorless ascospores (Plate VII, 10) which are shaped something like a section of an orange (Plate VII, 11) and measure about 5 by 1.5 μ . The asci early disintegrate leaving the ascospores free in the perithecial cavity (Plate VII, 9). Ascospores do not mature simultaneously in all the asci of a perithecium and in the same perithecium one may find mature spores free from the ascus as well as asci in which there is not yet any differentiation of ascospores. The ascospores are discharged through the neck canal (Plate VII, 9) and form a droplet (Plate VII, 2) supported by the fringe of cilia at the apex of the neck (Plate VII, 3).

The germination of the ascospores is similar to that of the coremial spores, and like them is not always constant. They may also become mixed with the *Cephalosporium* type of spores so that identification of the spores under the microscope is necessary in order to be certain that it is the germination of the ascospores. No budding of the ascospores was noted. They form a germ tube which early forms conidia (Plate VII, 12) and these conidia may bud like those of the *Cephalosporium* type.

ADDENDA

Letters from European Scientists Concerning Graphium, etc.

Having learned much from European literature, the writers thought it desirable to obtain more recent experiences and opinions of some of the scientists who have investigated this trouble from various points of view. So, in December, 1934, we selected the names of some thirty-odd investigators, covering each of the countries from which the disease had been reported, and wrote them the following letter. The answers received, (with such omissions as are not relative to the disease, etc.) are also appended.

We are indebted to the department's secretary, Mrs. Lillian D. Kelsey, for translations of the letters which are not included in quotation marks. In several cases more than one letter was received from the same country, and these are placed together for convenience.

Permission to publish was granted by the authors.

Connecticut Questionnaire

New Haven, Connecticut,
Dec. 6, 1934

In the autumn of 1933, there was found the first specimen of Dutch Elm Disease, *Graphium ulmi*, in Connecticut. During the year 1934, fifty-six other diseased trees were found here—one of them at a considerable distance from the infected area near the New York state line from which the fungus evidently entered our state. Many persons believe that the trouble will spread through Connecticut to the rest of the New England states if not stopped by us. With our apparently small invasion there is a better chance to control, or possibly eradicate it, in this state than in either New York or New Jersey where the fungus obtained a much larger invasion of the elm and a wider distribution. The writers wish to learn more about this disease in Europe, from those who have had personal contact with it, and for this reason we are asking a written response to the following questions:-

1. Where and when did it appear, and how seriously and generally has this trouble occurred in your own country?
2. How rapidly has it spread where found and is it likely to kill all of the elms of your country?
3. Have any extensive measures been used to eradicate or control it, and if so how successful?
4. Do you believe the complete destruction of infected trees (the only control measure now being used in the United States) to be effective, and if so on what basis or experience?
5. Are there any other troubles (such as other fungi, bacteria, insects, drought and winter injuries) that are serious or in part account for the injury and death of elms in your country?
6. Is there any indication that this trouble is on the decline in any locality and for what reason?
7. Is there any evidence how the trouble first came into your country or from where it first entered Europe?

Replies

Baarn, Holland,
April 11, 1935

"1-2. The first cases in Holland were stated in Hoeven and Tilburg in 1919 (Province Noord-Brabant, near the Belgian frontier). A year later it was found in various parts of Holland (a.o. Rotterdam). Still a year later, in 1921, it was found in every province of Holland. Before long, it was distributed generally in the South, the East and the center of the country. Its seriousness varies. In the South and East it is very serious (for so far there are still elm trees left); in the northwestern parts it is not quite as bad, though it is spreading continually. It seems not impossible, indeed, that this disease will kill all our elms.

"3. In Holland it is compulsory for owners of elm trees to remove badly diseased and dead elm trees, as designated by the State Forestry Service. The felled trees must be kept immersed in water, or their bark must either be removed and burned or covered with rough carbolineum. Since this practice had been started only when the disease had spread considerably, it was not possible any more to eradicate the disease. We believe, however, it is the only way to impede its spread as much as possible and to protect those areas where the disease has not yet appeared.

"4. In a country where the disease has just made its appearance, the destruction of infected trees is the best way to try to eradicate it. As a rule, though, felling the trees and removing and burning of the bark (even from the branches and the larger twigs) will do. The principal thing is to inhibit the bark beetles to brood in the infected trees. It is to be taken into account that *Scolytus multistriatus* may brood in relatively small twigs.

"I may add that it is my personal opinion that it will not be possible to eradicate the disease in the United States. By adequate control it may be possible, however, to delay the appearance of the disease in the northern parts of New England for some time.

"5. The answer to this question can be a brief 'no'. The occasional occurrence of *Polyporus squamosus* plays no part in this respect.

"6. So far as we know, the only reason for the disease being on the so-called 'decline' in a certain locality is that it killed all the elms present.

"7. We have no evidence regarding the provenience of the disease. It may be that its occurrence is due to an intensification in virulence of the fungus. On the other hand we can just as well assume that it has been present for a long time in a country without bark beetles, where it had little opportunity to spread. One can imagine, for example, that it has been present in Ohio for some time without being of any importance, just as it is now. In the war, when so much American material came to France and Belgium, the fungus may have been transported to Europe, where it spread quickly on account of its association with the bark beetles. This is a mere theory; to all probability we shall never know where the disease came from. The first reports of the disease in Europe are from the Western coast of the continent and it has spread from there."

Christine Buisman.¹

Malang, Java,
July 21, 1935

"You understand that I have not studied the Dutch elm disease after my departure to Java. I also cannot give you much more information than you can find in my publications (4, 5).

"I think that the Dutch elm disease is already a long time in Europe. Naturally it is impossible to prove this exactly, because it is impossible to isolate the Graphium out of old dead wood. The description of Dudley, [*Philos. Trans.*, London, 24: 1859-1863], 1705, makes it probable that already in that year the Dutch elm disease was in England. Over the old outbreaks of the Dutch elm disease in the Netherlands Mrs. Spierenburg gives in the same publication of the *Plantenziektenkundige Dients* very interesting information.

¹ We are sorry to record the death of Doctor Buisman which occurred March 27, 1936.

"I draw your attention to my conclusion on page 12 of my publication (*Med. Pltnz. Dnst.*) 'It is also evident that external circumstances have an important influence on the intensity of the attack of *Graphium*.'"

J. G. Betrem.

Nancy, France,
Dec. 17, 1934

The disease of the elm due to *Ceratostomella* (*Graphium*) *ulmi* is actually much developed in France, and it is feared that the greater part of the elms, according to the conditions in which they are found, even in the forest in the best ecologic circumstances, will disappear. The propagation of the disease was somewhat slow at first, but appears to have become more rapid in later years. It is undoubtedly true that the disease came from Belgium. It appeared first in the north of France, then extended in a general southwestern and southeastern direction, and at present is everywhere.

There have been no general measures taken for the control of the disease, nor for the destruction of the diseased trees.

I believe that the complete destruction of the trees at the first manifestation of the disease might be an efficacious remedy. It must be remarked, however, that this plan is exceedingly difficult but up to now no experience has been sufficient to decide upon this plan.

It appears incontestable that the *Scolytus* plays a rôle in the propagation of the disease. These insects contribute largely to the disease and death of trees attacked by the *Graphium*. In certain cases, also attacks of the *Galeruca*, causing more or less defoliation, tend to weaken the tree and favor attacks by the fungus.

There is no indication at the present time that the disease is declining.

It would seem that special attention should be paid to the degree of resistance of different species of elms. In regard to this it appears at present that *Ulmus campestris* is the most susceptible species, since it is attacked under all conditions, even in the forest. On the contrary, *Ulmus montana* appears to be more resistant, and no diseased specimens of this have been found even in the forest. The types of elms cultivated in France are often hybrids of *Ulmus campestris*, and these already show more resistance. They are attacked, but in different degrees. It therefore seems best to study the varieties in order to cultivate the elm in spite of the disease, the disappearance of which now cannot be counted on. The Asiatic species, *Ulmus pumila*, also shows a resistance beyond doubt. This resistance question has been studied notably in Italy.

Philibert Guinier.

Gembloux, Belgium,
Dec. 27, 1934

The disease appeared about 1919 in the northern part of our country, and very rapidly extended toward the south. In the region situated north of the Sambre-Meuse line, about 50 per cent of the elms are dead. In the region south of that line the number is between 10 per cent and 50 per cent.

No control measures have been taken.

Taking down the diseased elms may retard somewhat the propagation of the disease, and it is very possible that if this measure were adopted at the very first symptoms of invasion, it might arrest it.

No other disease or injury to elms exists with us.

In certain regions of our country the disease seems to decline. It seems that the surviving elms are more resistant.

P. Manil.

Geisenheim on Rhine, Germany,
Dec. 18, 1934

In response to your letter of the 17th of last month, I am pleased to inform you that we have done a large amount of work here on the elm death, *Graphium ulmi*.

A publication (37) which will answer your questions is about to be issued by the *Zeitschrift für Pflanzenkrankheiten*, and upon its appearance a separate will immediately be sent you.

G. Lüstner.

Kitzeberg, Germany,
Apr. 10, 1935

I undertook investigations on the Dutch elm disease, "elm death", only when the first outbreaks of the disease occurred in Germany and have worked upon it but in a secondary way.

I have set forth my observations in the following three articles:

- (1) A wholesale death of the elms in Germany. *Nachr. Deutsch. Pflanzensch.* 4: 84-85. 1924.
- (2) The Elm Death in Germany. (*Mitt. Dendtol. Ges.* 1924: 284-288.
- (3) On the Elm Death. (*Gartenschönheit* 1927: 165-166.)

The answer to your questions you will find partly in these articles, of which, unfortunately, I have no separates except for the first which I am sending you, and in publications of the following German authors: H. W. Wollenweber, C. Stapp, H. Richter. (*Biologische Reichsanstalt* Berlin-Dahlem) and G. Lüstner, Geisenheim on Rhine, (*Lehr und Forschungsanstalt für Gartenbau*), where you may find answers to your questions, in case you have not already obtained separates of these articles.

H. Pape.

Tharandt, Germany,
Jan. 14, 1935

1. The elm disease was first known to me in Dresden in the year 1928, following the outbreak of the disease in my own garden. In the next spring I proved that the disease had broken out in the environs and asked that measures be taken against it. However, as this was not according to the practice, nothing was done. The disease in the fall of 1929 had made heavy advances; by my insistence it came to the attention of the Ministry of Economics of Saxony. I might say that a year or two before, a case of epidemic elm disease was reported by Dr. Munch in Dresden, and the tree was destroyed.

2. I can give no information as to the rapidity of the spread of the elm disease, as I personally know nothing about its spread, only of the appearance of the very destructive disease. In any case its spread in Saxony is very serious. My opinion is that the disease will, in time, be eradicated when the diseased elms have been felled, and those less badly infected have been protected by some control measure. However, other injuries to elms, for example, *Psylla ulmi*, should be controlled in order to avoid weakening of the elms and thus lessening their susceptibility.

7. As to the introduction of the disease into Saxony I have no information, and in regard to its introduction into Europe I have no knowledge except what has been written on the subject.

H. Prell.

Berlin-Dahlem, Germany,
Jan. 24, 1935

The Elm disease, *Graphium ulmi*, appeared somewhat in Germany in the year 1924. It originated, as far as it is known, in France where it appeared in 1918. The disease has comparatively rapidly spread from the west over mid-Germany, while the north-eastern part (East Prussia, Pomerania, Mecklenburg) as well as the greater part of South Germany have escaped.

The only control measure known up to now is the unsparing destruction of diseased trees. Experiments in the infected forest stands have shown that while the disease is not entirely eliminated by the immediate cutting down of the trees, yet in economic situations this plan can be followed. Investigations are being made to determine whether elms, planted in cities or streets as ornamental trees, can be grafted with less susceptible varieties, as *Ulmus pumila* and *U. pumila* var *pinnato-ramosa*. These experiments are still being carried on.

Naturally there are cases in which elms die from other causes than *Graphium ulmi*, such as drought, frost, Hallimasch attack, etc. These, however, play no practical rôle.

J. A. Richter.

Munich, Germany,
Jan. 1935

"The Dutch elm disease was first reported from the Netherlands in 1919. A few years later it appeared already in the north of France and the northwest of Germany, in 1927 in south Germany, 1930 in Italy and Austria.

"It is unknown how it came into the Netherlands, perhaps together with timber, as was the case in America.

"If never killed *all* the elm trees; sometimes the progress of the disease is a rapid one, sometimes it takes a long time. In our country it is impossible to destroy all the infected trees completely.

"The felling of the trees is not a really successful remedy, but it certainly helps to keep back the catastrophe. If you want to fell the trees, do so in winter and burn every bit on the spot. It is also useful to close the wounds on branches and stems with coal-tar. Bark beetles do not always account for spreading the disease."

Professor von Tubeuf.

Imperial Mycological Institute,
Kew, England,
Dec. 17, 1934

"1. The disease was first observed near London in July, 1927, and in the following year was found to be well established in a large part of the south, east, and center of England. By 1931 the total number of counties infected was 38, the north and west being still free. An historical survey of the disease in England is contained in Peace's paper in 1932, abstracted in the *Review of Applied Mycology*, xii, p. 334. The latest reference to the present position of the disease in Great Britain is contained in the Tenth Annual Report of the Imperial Forestry Institute, University of Oxford, 1934 (received by us last month) in which it stated that no appreciable increase has occurred in the year 1933-4 and no disease appears to have been found in Scotland. It is quite certain that the first infections must have occurred before 1927.

"2. The rapidity of spread has varied considerably and has frequently been slight. At Kew, for instance, the first cases seen in the Royal Botanic Gardens several years ago (1928 or 9) have not been followed by others. It is impossible to say whether it is likely to kill all the elms in the country, but so far the indications are to the contrary.

"3. No. Eradication on a really large scale has not been practiced, but in certain valuable collections or in some private estates eradication has been tried. It is impossible to say whether it has been effective in view of the erratic behaviour of the disease in England.

"4. No views, except that if taken early enough and thoroughly done it ought to be effective.

"5. Several other elm diseases have been found during the survey and some are liable to be, and have been, confused with the disease caused by *Ceratostomella ulmi*.

"6. The numbers of recoveries reported by Peace in the above cited paper are considerable. Whether these are permanent is not known.

"7. The early distribution of the disease suggests that it reached England from the Continent of Europe. The early and heavy infestation of parts of the County of Essex may point to an introduction from the Netherlands.

"I have long thought that a possible explanation of the appearance of the disease in Europe may be sought in the congregation of peoples from the Far East, especially Chinese and other Asiatics, in the Labour Corps recruited by the Allies for work behind the lines during the Great War. Such peoples often carry their possessions in fibre or rough wooden containers. The observations of Guyot in northern France in 1918 (see R.A.M., p. 334; xi, pp. 551, 610) appear to me to have a special significance in this connection. A. Puttemans in a little pamphlet, '*Notes Phytopathologiques et Mycologiques*', dated Bruxelles, Novembre, 1918, and evidently written during the German occupation, mentions in a few lines a disease of elms (*U. campestris*, *U. montana*) in wayside trees of the township of Brussels prevalent since several years and causing the death of numerous elms of all ages. It appeared to spread through the soil. This area would be out of reach of labour corps contamination but the disease may well have been different."

E. J. Butler,
Director.

Imperial Forestry Institute,
University of Oxford,
April, 1935

"1. The disease was first discovered in this country at Totteridge in Hertfordshire, not far north of London, in 1927. In 1928, when a three months survey was undertaken, it was found in nineteen counties. It is therefore almost certain that it was in this country before 1927. From 1928 to 1931 it spread considerably, and in certain areas became serious. In 1932 there was a big reduction in the severity of the disease, a large number of trees making a temporary or permanent recovery (see 6). Since 1932 it has again increased and is now not far from the 1931 level. It is roughly speaking most severe in the eastern counties; most of the areas where it is common lie to the southeast of a line drawn from Dorset on the south coast to the Wash. North and west of this line it decreases in intensity, and has not so far been found north of Wigan in the west and Durham in the east of England.

"2. The rate of spread of the disease is very varied. In certain areas it has made little advance since it was first reported, in others its progress has been comparatively steady, while in others years of rapid advance have alternated with years in which the disease has made little progress. In a few limited areas the number of trees infected and dead may be as high as 80 per cent; but figures ranging from 5 to 30 per cent infected and dead are much more frequent, and outside the area where it is common, mentioned above, 0 to 5 per cent infection would be more usual. At the present rate of spread it will be a very long time before all the elms are killed. Indeed, were a reasonable number of elm suckers, which occur so abundantly in our hedges, allowed to grow up into trees, it should prove possible to replace the losses due to the disease. On the other hand, if the fungus were to become virulent in all the trees in which it is in a dormant or semi-dormant condition (see 6), the losses would be enormous and irreplaceable.

"3. In 1927 and for a short time in 1928 we advocated cutting down and burning infected trees. But the elm is the commonest non-plantation tree in many parts of this country, and we were soon met with refusals and requests for compensation. Nor was it possible to provide the staff necessary to supervise such measures. In view of the fact that the fungus exists in many trees that show only the scantiest outward signs of the disease, and probably in a great many that show no signs at all, I am strongly opposed to any policy of eradication being tried again in this country.

"4. As stated above, I do not favour any attempt at eradication in this country. To remove all elms showing external signs of the disease would mean the removal of many elms which are now, and may remain for years, in a comparatively healthy condition. And even then the disease will probably remain in apparently healthy elms. (*Ceratostomella ulmi* has been isolated from several elms showing no external signs of disease). In any case a policy of eradication is only justified if the number of trees removed in a given period exceeds the number of new cases.

"5. In Scotland and in the north and west of England, mostly outside the Dutch Elm Disease area, the older elms are frequently found to be in a debilitated condition. Though death seldom seems to occur, the general health of the elms is probably worse than in the south. The symptoms differ from those of the Dutch Elm Disease, the

dieback being more gradual, and internal markings being more or less absent. Several fungi have been isolated, and work on them is now in progress. I think that the Elm Bark Beetle, *Scolytus scolytus*, is capable of causing the death of elms already weakened by drought, but this type of damage is not very common. The beetle is nearly always found in association with *Ceratostomella ulmi*. I cannot find that anything acts as a predisposing factor to this fungal attack, if infection by agency of the beetle be excepted. Trees of all ages and in all situations seem liable to attack. It seems likely that the trees are usually infected by the beetles in the course of their summer feeding, and the beetles seem to have no strong preference as to the type of elm on which they feed.

"6. I have mentioned the peak year in 1931 above. Assuming that *Scolytus* beetles are the main agents of dissemination of the disease, I had thought that this peak might have been due to an increase of the beetle population in 1930 and 1931, owing to the large number of elms blown down in the gales in the autumn of 1929, these trees providing increased breeding grounds for the insect. But this is pure assumption. The decrease in 1932 is harder to explain; it has been found in all years, but particularly then, that trees make a temporary recovery, the fungus still remaining alive in them. Occasionally these recoveries become permanent, the fungus dying out; more often the disease breaks out again, or remains dormant. One elm is known which last showed external symptoms in 1928, but in which the fungus is still alive. It is hoped to find by inoculation whether the fungus has lost its virulence. The fact that these trees may remain for years in a semi-diseased state is another argument in favour of a policy of *laissez faire* with regard to control.

"7. There is no evidence as to how the disease entered this country. Since the disease was first discovered in Europe, there is obviously no information as to how it reached that continent. There seems every possibility that it may have arisen as a mutant in Europe, or that some peculiar climatic circumstances were needed to set it on its present career."

T. R. Peace.

Rome, Italy,
Dec. 24, 1934

1. I, myself, found the disease for the first time in Italy in June, 1930, on material from the province of Plodena (Emilia), and also almost at the same time recognized the disease on material from the province of Ravenna (Emilia). In Emilia the injuries are and have been from the first year (1930) notable in all localities, and in some localities as many as 50 per cent of the trees are diseased. However, the disease may have been present for a couple of years before it was discovered.

2. The diffusion of the disease from Emilia proceeded toward the south, perhaps also toward the north; however, elms are much less common in the north. It was observed in Emilia in 1930; appeared in the Marches and in Tuscany and Umbria in 1931 and in the Abruzzi, and perhaps also in Lazio, and in 1932 it was already quite common in Lazio and in the Campagna. It certainly existed in Lombardy, but I do not know how long it has been there, and it is not widely diffused because the elm is not cultivated. As to the rest of Italy, Sardinia and Sicily, I have no information.

3. Not much has been done on the part of the State to control this disease, though some control has been privately done. It should at all times be considered that cutting down or isolating the trees is a control measure. Land owners have been advised to replace their dead elms, and for this purpose the planting of *Ulmus pumila* var. *pinnatiramosa* has been counseled. Those who have attempted to cure trees not too gravely attacked have cut off the diseased limbs up to the healthy wood, and then disinfected the wood with iron sulphate, covering the cuts with wax. This has been useful in disinfecting and cleansing the trunks. The trees so treated have done well for about two years, but are always subject to new infection, especially when in the vicinity of large areas of infection.

4. The destruction of the diseased trees is not feasible because in many regions *Ulmus campestris* is widely diffused owing to its use as a protection for the vines.

5. Other deadly diseases of the elm are not present in Italy. To a certain extent there is the mucous flux, and attacks of *Galerucella*. In the reports of the death of the elm there are the Scolitidi, which represent the principal carriers of the disease.

6. It does not seem that there is a decline in the death of the elm, although in a Congress held in March of this year at Forlì to discuss the means of control against this disease, I had occasion to hear from the Directors of the Traveling Scientists, that the number of new diseased elms in the last two years seemed to be less than in the preceding years. This concerned chiefly the region of Emilia and the Marches. The reason is not known.

7. It is not possible to state how this disease was introduced into Italy, but probably it entered by means of plants brought in from abroad. Its first appearance in Europe was in Holland.

Cesare Sibilìa.

Bologna, Italy,
Dec. 19, 1934

"With reference to your letter of December 6, 1934, I can suggest you that the response to all the questions concerning the Dutch elm disease shown in your letter will find a response (for my own land) in my two papers [nos. 23, 24 given in our bibliography.] I have demonstrated on new basis the hitherto unknown importance of this Coleopteron [*Scolytus sulcifrons*] in the multiplication and spread of the disease."

Athos Goidanich.

Bologna, Italy,
March 30, 1935

"Before giving you an answer I desired to consult other Italian pathologists who have studied and dealt with this disease. All the inquiries were immediately translated and submitted to them. Today after a few weeks I am already in possession of several answers which may be condensed as follows:

"Prof. A. Draghetti, Director of the R. Stazione Agraria Sperimentale, Modena.

'1st. The first localities infected by the Dutch elm disease were discovered during the summer of 1929 in accordance with my own observations on the plains and hills near Sassuolo, Castelvetro and Rubiera (20 miles west of Bologna). Almost simultaneously the disease was noticed at Ozzano-Mirandola and farther away at Rimini (about 65 miles east of Bologna) on the Adriatic coast.

'2nd. The spread of the disease was very rapid at first, from 1929 to 1932. In 1934 the disease seems to have lost its original virulence but it is still spreading and the trees attacked are being killed. At first it took two or three years to cause the death of an infected tree, whereas at present the destruction is slower.

'3rd. The removal of dead branches from infected trees has so far given no great results.

'4th. Judging from the actual progress of the disease it would not seem that the Dutch elm disease should kill all infected trees within a short time.

'5th. Damage to elm plantations caused by drought, dampness, rot, etc. are in close connection with the unsuitableness of the soils which are too heavy.

'6th. Great damage to the production of leaves and to the general healthiness of trees has been caused by the *Galerucella luteola* Müll, which in certain years destroys the entire foliage of the elm trees.

'7th. See points 2 and 4.

'8th. The disease was first noticed after the terribly cold winter of 1928/29 and was reported as a result of the exceptional frosts endured. Up to 1929 the percentage of trees killed by the Dutch elm disease was between 2 and 5 per cent; after that year the disease spread very rapidly and attained annually 10 to 15 per cent.'

"Dr. Gabriele Goidanich, R. Stazione di Patologia Vegetale, Rome.

'1st. The first notice of the appearance of the Dutch Elm Disease in Italy is that of Prof. C. Sibilìa, who discovered cases in June of 1930 in the Province of Modena.

'2nd. Shortly after, the disease was found almost simultaneously in many other districts even fairly distant from the first center. This does not mean, however, that a rapid spread of the disease took place. The fact of the declaration being contemporaneously made in various localities is owing to the scare produced by its appearance so that every one hastened to examine his trees and to perceive infected areas.

'Whether the disease will kill or not kill all our common and white elms (*Ulmus campestris* and *U. americana*) cannot at present be said, as a certain lapse of time is necessary to judge to what extent the ravages may extend, but according to the present situation no great hopes are to be entertained.

'3rd. Up to now official measures to control or eradicate the disease have not been taken as far as I know. For the moment there exists but the suggestion made by my cousin (Prof. Athos Goidanich) and myself and those made at the meeting held at Forlì in the spring of 1933.

'4th. The total destruction of all infected trees is undoubtedly of great advantage if carefully and thoroughly performed.

'5th. The genus *Ulmus* is not affected by other diseases of practical importance. Sometimes insects may damage elm trees by gnawing the interior part of the trunk.

'6th. There is no indication of diminished virulence of the Dutch elm disease nor have we any means of knowing whence and how the *Graphium* (*Ceratostomella*) *ulmi* first entered Italy.'

"Hon. Vittorio Peglion, Director of the R. Instituto Superiore Agrario, Bologna.

'Since the few cases discovered which indicated the appearance of the disease in the neighborhood of Modena as early as 1929, in the course of a few years we have arrived at a veritable hecatomb especially in the territory of the middle hills and in the plains which, forming a line with the river Po and the Appennine System (Mts.), includes lower Emilia and Romagna as far as the sea. The investigations made without interruption since 1929 have confirmed the identity between the form of disease spreading also in central and southern Italy and the form described by Dr. Schwarz as early as 1919.

'The infection begins with a phenomenon of withering of one or more branches, either lateral or in the crown of the tree, which are noticed by fits and starts along the rows early in June. At the top of a beautiful green elm tree rises a branch with the leaves faded as if it had been blighted, writes Alfred Panzini. The doom of the tree which is often secular is sealed. During the following year the withering has become general and often in the third year the tree is hopelessly compromised. The cause of the infection is a fungus, the *Graphium ulmi* isolated by Dr. Schwarz, whose relation with the *Ceratostomella ulmi* has been determined by Dr. Buisman.

The control of this truly disastrous calamity appears practically rather uncertain. The use of insecticides and insecticide substances against the carrier (*Scolytus*) is almost impracticable. Whence even the entomologists limit themselves to advising the felling of infected trees at the beginning of spring, immediately barking the trees and burning in the place the bark which harbors the insects. Neither is it possible to act directly against the *Graphium* (*Ceratostomella*) *ulmi* by means of anticroptogamic treatments.'

"Prof. Dante Longhini, of Suzzara (Mantova) writes as follows:

'The *Graphium* (*Ceratostomella*) *ulmi* appeared in this district in 1931 while in 1932 it was much in evidence. In 1933 it continued to spread with much virulence, while in 1934 the disease did not spread so greatly as in the previous years. No specific control measure has been tried and none is believed to have any efficacious effect unless it be the planting of *Ulmus pumila* which here gives great promise; indeed they are very good. Among the other troubles of the elms we notice the *Gallerucella*; whereas the drought maintaining slow growth, contributes to the development of the Dutch elm disease.'

'All these reports about the Dutch elm disease agree with each other and also with what I have written in my book "The Dutch Elm Disease and the Introduction and Cultivation in Italy, of the Siberian Elm (*U. pumila* L.)" Edizioni Selsa, Bologna, 1934, pp. 119 with 24 plates.

'Herewith I epitomize what I have written in the book itself on the points about which you inquire:

"1st. The first cases of the Dutch elm disease were found in June, 1930, on specimens collected in the province of Modena; but also in the previous year (1929) some elm trees in the said province were infected.

"2nd. After the discovery of the infection in the province of Modena, cases were here and there discovered throughout the whole length of the Emilian Way (State Route No. 9).

"3rd. The system of parallel cultivation of the elms and the vines renders it more difficult to make use of any controlling measure such as disinfection, uprooting and barking. In this direction only a few experiments have been made from which nothing has resulted to encourage further attempts.

"4th. The total destruction of the infected trees in order to eradicate the disease is not with us desirable, since it means at the same time the total destruction of the vines which are supported by the elm trees, cultivated between the river Po and the Appenines. In the same way, in the control of the disease, it is not possible entirely to remove all infected trees. This does not apply to the elms cultivated without the vines along roads or in parks where they are easily surveyed, such as we meet in the vine districts and in the woods.

"5th. During the last few years we have had invasion of *Galerucella*, an insect which devours the leaves. These insects, by stripping the trees of leaves in these years in which the invasion is intensive, deprive the peasants of the advantage of using the leaves as food for cattle. We have also some *Scolytus* (Bark Beetles) and grubs which are harbored by the elms but seldom cause mortal damage. It is estimated that in half a century of life in rows, the elms may lose in consequence of the attacks of these pests, about 5 per cent of their number and only when they are planted in unsuitable soil. In these cases the common elm (*U. campestris*) outlives even the attacks of this vermin.

"6th. There is no indication that with us the disease is diminishing its destructive activity. There have been noticed in certain districts with 70/80 per cent of dead or dying trees and others with only 8/10 per cent in the same conditions. It is supposed that the districts spared by the disease are those where the bark beetle is less prevalent.

"7th. Noticed for the first time in Holland, this microscopic fungus spread little by little into the other states of Europe with a rapidity such as to give rise to the supposition that it was carried by the wind. This disease has spread with such lightning rapidity as to have no parallel on record with regard to the general diseases of plants."

Arturo Ansaloni.

Geneva, Switzerland,
Dec. 24, 1934

In reply to your letter of Dec. 19, I am enclosing herewith a fascicle. In response to your questions I reply as follows:

1. Found in Germany in 1918, I believe it spread to Geneva in 1922.
2. Rapidly enough, the greater part of our young trees are attacked.
3. Yes, cutting down the trees has been resorted to, also the tops of the diseased branches, and sprinkling (spraying) the trunks.
4. I do not fear that the disease will be general, but that the more resistant individuals can be saved.
5. Aside from the fungus, I have observed bacteria, and also *Scolytus* and other insects.
6. No, the disease has much more the tendency to increase.
7. The disease was reported in Europe in 1918, but it is probable that it appeared earlier.

Prof. Alfred Lendner.

Zürich, Switzerland,
Jan. 12, 1935

In reply to your honored questionnaire concerning *Graphium ulmi*, I regret that I can only say that neither the specialists of our High School, nor I myself, have had practically anything to do with this disease. The disease up to now has not been

brought to our attention, and owing to the limited presence of varieties of elms in our forests, it is scarcely likely that a great outbreak can occur. I refer you to the publication of Arturo Ansaloni on 'The Death of the Elms and the Diffusion in Italy of the Siberian Elm', issued at Bologna in 1934.

H. Badoux.

Prague, Czechoslovakia,
March 12, 1935

To your questions of Dec. 6, 1934, addressed to Dr. A. Nemec, Prague, we would say that today we have sent you a separate (Bull. by Kalandra & Pfeffer, 1935) on *Graphium ulmi* as this work was still in the printers' hands. In the German conclusions you will find the answers to your questions 1 to 7.

State Inst. Forest Research.

Prague, Czechoslovakia,
Jan. 10, 1935

"1-7. This fungus has been widespread in Bohemia, Moravia and Slovakia according to my special investigations (in the under Carpathian Russia I didn't make any thorough inquiry). They started 1931, when I was charged with the phytopathological research and teaching in the school of Agriculture and Forestry in the Czech Technical University of Prague and after I had been introduced to the study of this disease during my journey in Holland, Belgium and France. When this disease did appear in our country nobody correctly knows. Some foresters say that it has been observed after terrible frosts 1928-29 (until -42°C.) But it must have spread much earlier because there are found trees of 60-80 years destroyed from it. I suppose that those frosts have contributed to the great spread of *Scolytus* which generally accompanies the diseased trees (*Ulmus campestris*, *U. effusa*, *U. montana*) in our country too. The *Graphium* disease is spread all over our country through all greater parks in bigger cities (Prague, Brno, Niba, etc.), where the elms are planted in greater numbers close together. There seem to be hotter and drier districts that succumb to epidemics especially.

"But the fungus is able to attack our forest with mixed foliage trees too. I noticed at Poděbrady (a resort place in Prague) 14 per cent contamination of young trees in one of them, in another case 90 old trees destroyed by it. Another wood in Prague had to be cut before the proper time, 400m³, 40 wagons timber.

"In the parks of Prague have been cut 330 trees of different varieties of *Ulmus*, but there remain many other hundreds of diseased trees.

"2. In an avenue of Poděbrady I observed in 1932 from 55 elms 2 diseased in a high degree; 1934, 18 very sick, 4 of them with dead crowns. The disease spreads further on quickly. Nevertheless there remain still many trees healthy—how long nobody knows.

"3. One doesn't do so much against the disease in our country, the authorities of our cities do not bother themselves with science, although one knows that in Prague the disease has been spread a great deal by grafts imported from Holland. We only ceased to plant elms in some parks. There is no quarantine law against *Graphium* with us.

"4. I believe the complete destruction of infected trees is effective. Then the pruning of the sick branches can enlarge the infection in consequence of regeneration of sleeping buds which at once acquire the infection. The sick trees are soon invaded by *Scolytus*. I have isolated very easily the *Graphium* from their pupae, from their excrement, even from ants circulating near the sick trees.

"5. In many cases of *Ulmus montana* which showed external symptoms of the *Graphium*, I was unable to isolate this fungus, but other fungi which I did not get any time yet to test. In one case, *Ulmus campestris*, I isolated only *Verticillium Dahliae*, in another case *Verticillium Dahliae* and *Graphium ulmi* from the same sample. This seems to be of some importance with regard to apricots which in our country are heavily

infested by *Verticillium Dahliae*. Moreover *Graphium ulmi*, *V. Dahliae* and *V. albatrum* seem to me not so morphologically different as one otherwise believes them.

"6. There is no decline of this trouble in our country."

Jaroslav Peklo.

Bucharest, Roumania,
Dec. 22, 1934

"1. The Dutch elm disease was noted for the first time in Roumania in 1929. We could not tell where it first appeared, because it was noted at the same time in different districts quite far situated one from the other. The attack was quite serious, all our varieties being attacked to the same degree. The trees, which were situated in humid regions or near a river, suffered less from the attack.

"2. The disease has spread very rapidly. We do not have any region free from the attack, and in many places the elms were completely destroyed. About 70-80 per cent of our elms are killed.

"3. In some districts the trees were cut and burned but not systematically enough so as to be able to give any definite data.

"4. We believe that the complete destruction of infected trees could be effective, but we do not have any experimental data.

"5. We are of the opinion that the serious attack observed during the last few years is due, partly, also to the severe winter of 1929.

"6. We have no signs of the decline of the disease.

"7. We could not tell how the disease was introduced in our country. As mentioned above, the disease appeared at the same time in a few regions of the country, while all the neighboring countries were contaminated."

Prof. Tr. Săvulescu.

Zagreb, Yugoslavia,
Feb. 20, 1935

"1. I have observed the first diseased elm trees (*Ulmus campestris*) in the mixed stands of oak (*Quercus robur*), elm and ash (*Fraxinus excelsior*) in the vicinity of Zupanja, Slavonia, in the year 1928. In the following years the number of infected and killed trees was always larger and the isolations gave in all cases pure cultures of *Graphium ulmi* Schwarz. According to the opinion of the foresters that the bark-beetles (*Scolytus scolytus* and *Scolytus multistriatus*) are the cause of this dying away, there have been trials to control the disease using trap-trees, but the disease did not stop and was in further progress. In the year 1932, the disease was already widespread in the country and in the last two years we have had the occasion to state that it attacked the trees in towns and villages also. Only the elms (*Ulmus glabra* H.) in the mountain region are disease free at the present time.

"2. From the answer under 1, it can be seen that the disease spread almost throughout the whole country in six years and it is likely to kill a very large number of elm trees in our country.

"3. There have been trials to eradicate and control the disease in the towns, but to our greatest displeasure with very problematic success. The trials made in the woods are not yet as far, that we could be able to give you any answer.

"4. Whether the complete destruction of infected trees, the only control measure we know at present, will have the desired effect, is in our case difficult to say, because in some cases we started with the control somewhat late and in the others we are expecting the results of our control measures in the coming years.

"5. From all our investigations and experiences it can be clearly seen that the bark-beetles, *Scolytus scolytus* and *S. multistriatus*, play a quite important rôle not only in spreading the disease, but also hastening the death of the infected trees. In very many trees we have found the honey fungus, *Armillaria mellea* (Vahl) Fr., to be also a significant factor in the dying-process of the elms.

"6. There is no indication that this trouble is on the decline, but on the contrary it seems to be progressing in severity.

"7. There is no evidence how the trouble first came into our country. We do not import elm logs or living plants and as far as I could find out we did not import these in a single case, at least in the last 20 years. It may be that the bark-beetles were the carriers of the fungus from neighboring states (Austria) in which the disease occurred at an earlier time than in our country, or there may be some other, until now unknown, means of spreading the disease-causing organism."

Prof. Vladimir Skorić.

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EXPLANATIONS OF ILLUSTRATIONS

Plate I. Showing curled leaves caused by (1) *Cephalosporium* sp. and (2) *Graphium ulmi*.

Plate II. *Graphium ulmi*. Showing partially denuded top of tree (1) at Darien, Conn.; similar tree (2) at Montclair, N. J.; and group of infected trees (3) at Amsterdam, Holland.

Plate III. *Graphium ulmi* in Connecticut trees. (1) First tree found in Connecticut, at Glenville; (2) lane of trees at Old Lyme; (3) removal of first tree found at Greenwich.

Plate IV. Characteristic vertical tunnels in elm wood just beneath the bark caused by (1) *Scolytus multistriatus*; (2) horizontal tunnels on inside of bark caused by *Hylurgopinus rufipes*. These two insects are the known carriers of the Dutch elm disease in Connecticut. (3) Coremia of *Graphium ulmi* growing in a tunnel of *Hylurgopinus*; (4) details of (3) showing the black stalks and whitish spore masses of these fruiting bodies; (5) an isolated coremium.

Plate V. (1) Section of a twig infected with *Graphium ulmi*, showing the discolored spots in the wood. (2) Petri dish culture made from an infected twig, showing the characteristic growth of the *Cephalosporium* stage of *Graphium ulmi*. (3) Mycelium of (2), showing connecting hyphae. (4) Mycelium producing *Cephalosporium* spores. (5-8) Sections of wood showing strands of mycelium. (3-8) Show high magnification.

Plate VI. Different stages of *Graphium ulmi*. (1) Mass of viscid spores at tip of coremium; (2) cross section of its stem; (3) details of its tip showing fertile threads that produce the spores. (4) A short, thick coremium. (5) Details of coremium tip showing how the spores are produced. (6) Germinating coremial spores. (7) Staghorn bodies that frequently appear in cultures. (8) A sclerotium that frequently develops in cultures. (9) A cross section of a larger sclerotium showing the colorless interior. (10) An imperfect perithecium that appeared in some of the uncrossed cultures made from Connecticut material. All figures are magnified but (5-6) more highly than the others.

Plate VII. This gives details of the perithecial stage of *Ceratostomella ulmi* but of different magnifications, (10-12) showing the highest. (1) Shows a slightly magnified perithecium with its bulbous base and elongated neck. (2) A viscid drop of the ascospores at tip of a perithecium. (3) The crown of cilia at the tip free from the spores. (4) A cross section of the neck showing the hyaline interior. (5-6) Cross sections of immature perithecia with necks not elongated. (7) Cross section of a perithecium showing differentiations of ascogenous tissue and the central hyaline tissue in the broken neck. (8) Development of the asci within the perithecium. (9) Liberation of ascospores within the perithecium. (10) Asci and ascospores from an enlargement of (8), the arrow pointing to an ascus producing 8 ascospores. (11) The lunar-shaped ascospores free in the perithecium. (12) Germination of swollen ascospores.

Plate VIII. Perithecia of *Ceratostomella ulmi*, all about equally magnified, showing peculiar bodies whose functions were undetermined. (1-6) Show the enlarged bodies often found associated with the development of the ascogenous tissue and even in later stages with the asci; (7-8) the trichogyne-like thread (found in the vicinity of the neck of the perithecium) that sometimes ends in a bulbous base (3).



Leaves injured by (1) *Cephalosporium* sp., and (2) *Graphium ulmi*.



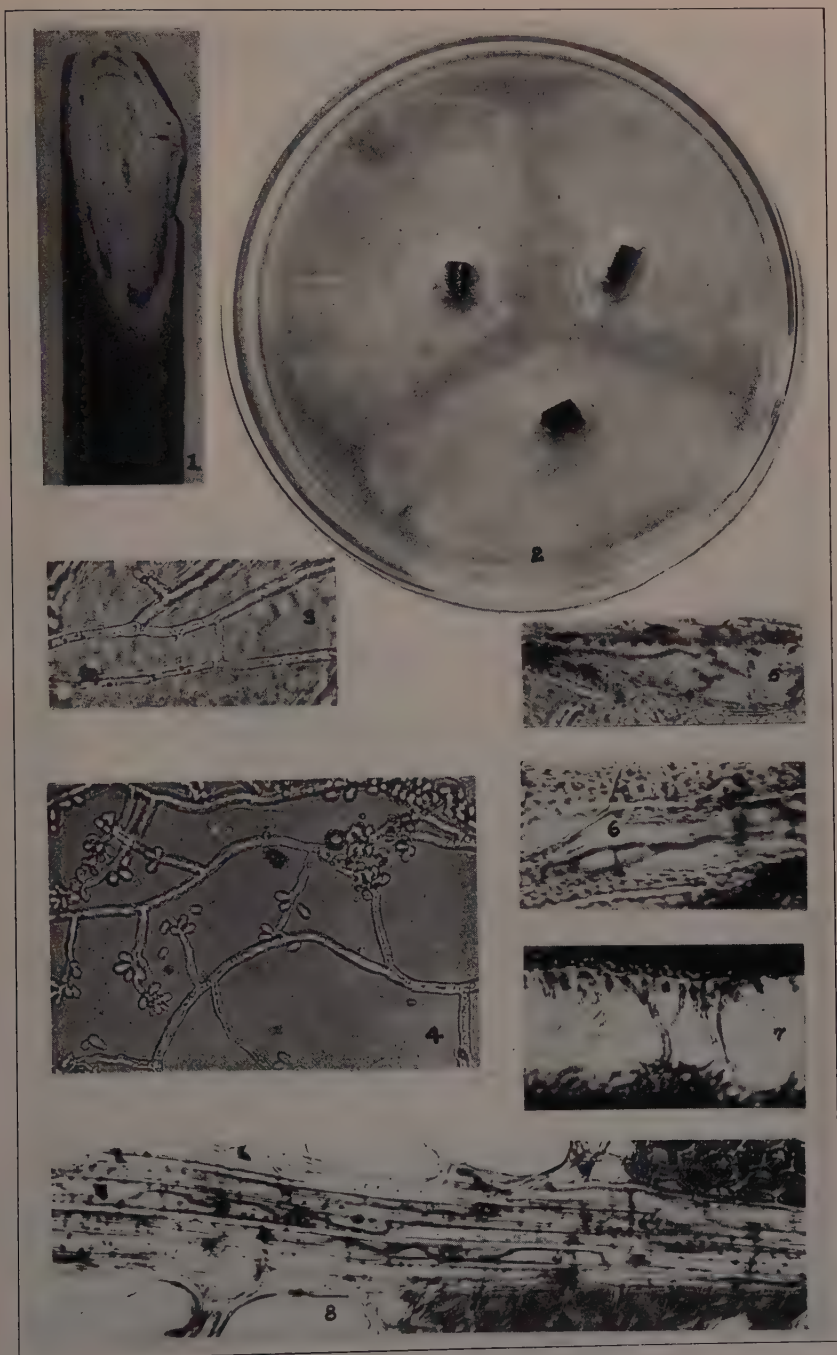
Trees injured by *Graphium ulmi*.



Graphium ulmi in Connecticut trees.



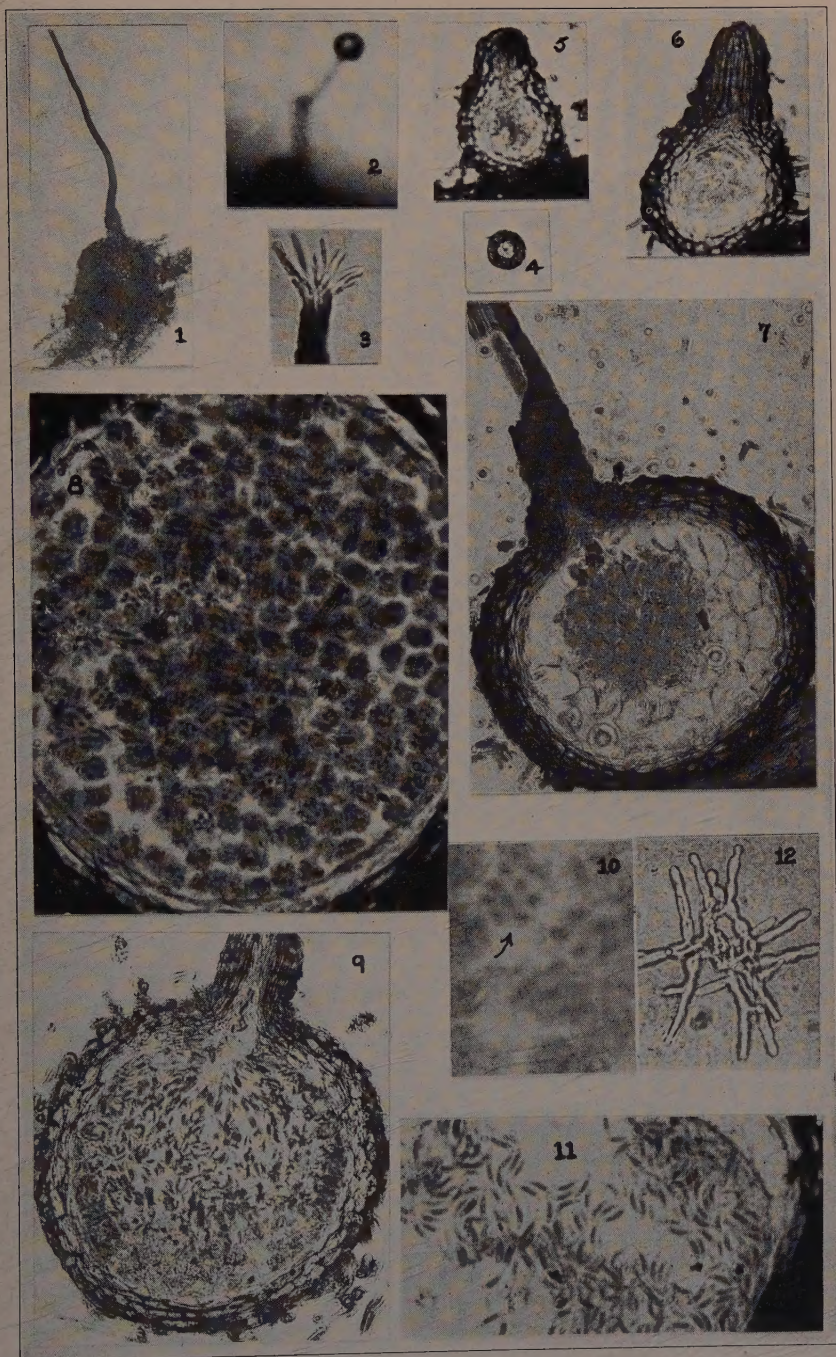
Insect tunnels and coremia of *Graphium ulmi*.



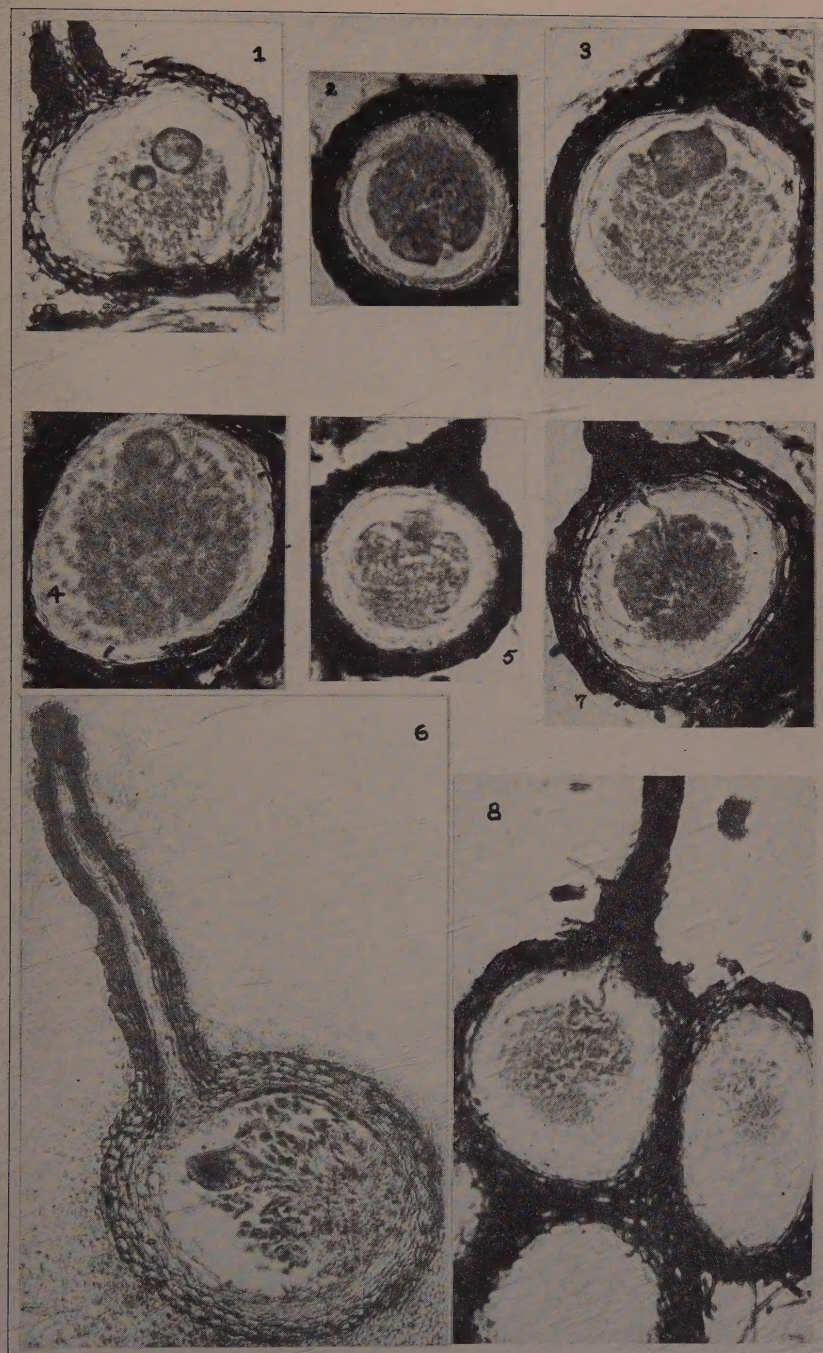
Cephalosporium stage of *Graphium ulmi*.



Coremia and other stages of *Graphium ulmi*.



Perithecial stage of *Ceratostomella ulmi*.



Peculiar bodies in perithecia of *Ceratostomella ulmi*.

